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(54) Title: DIFFERENTIALLY EXPRESSED GENES IN LARGE GRANULAR LYMPHOCYTE LEUKEMIA

(57) Abstract: The subject invention concerns gene sequences and the use thereof as markers for large granular lymphocyte (LGL) leukemia. The gene sequences of the invention are differentially expressed in LGL. Another aspect of the invention pertains to therapeutic compositions directed to gene expression and gene products of differentially expressed genes in LGL. The invention also concerns methods for screening and identifying compositions that may be of therapeutic benefit to patients having LGL leukemia and/or autoimmune disorders. In addition, because a large fraction of patients with T-LGL leukemia also have rheumatoid arthritis, these differentially expressed genes also represent novel targets for the diagnosis, prevention or treatment of rheumatoid arthritis and other autoimmune diseases.

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DESCRIPTION

DIFFERENTIALLY EXPRESSED GENES IN LARGE GRANULAR LYMPHOCYTE LEUKEMIA

This invention was made with government support under the Veterans Administration, grant number CA83947, and the National Cancer Institute, grant number CA90633. The government has certain rights in the invention.

Cross-Reference to Related Application

This application claims the benefit of U.S. Provisional Application Serial No. 60/319,910, filed January 28, 2003, which is hereby incorporated by reference herein in its entirety.

Background of the Invention

Large granular lymphocyte (LGL) leukemia is a human lymphoproliferative disorder often associated with autoimmune disease, such as rheumatoid arthritis. The etiology of LGL leukemia is not known. Large granular lymphocyte are a morphologically recognizable lymphoid subset comprising 10%-15% of peripheral blood mononuclear cells. LGL can be divided into two major lineages: CD3-negative cells (CD3-) and CD3-positive cells (CD3+). CD3- LGL are natural killer (NK) cells that mediate non-major histocompatibility complex (MHC)-restricted cytotoxicity and do not express the CD3/T-cell receptor (TCR) complex or rearrange TCR genes. CD3+ LGL are T-cells that do express CD3/TCR complex and rearrange TCR genes. A syndrome of increased numbers of circulating LGL associated with chronic neutropenia was first recognized as a distinct clinical entity in 1977. LGL proliferations are now known to be clonally derived from either of their counterparts (CD3- or CD3+ LGL). Although the etiology of LGL leukemia has not been fully elucidated, some evidence suggests that the initiation event may involve an HTLV-I like retrovirus.

Examination of the peripheral blood is critical for establishing the diagnosis of LGL leukemia. Characteristic features of the disease include larger than normal lymphocytes with abundant pale cytoplasm and prominent azurophilic granules. Patients with clonal CD3+ LGL (T-LGL) possess clonally derived lymphocytes with a CD3+, CD16+ and CD57+ phenotype. Autoimmune features are characteristic of this disease, and these patients

resemble that of Felty's syndrome and present with the clinical triad of rheumatoid arthritis, neutropenia and splenomegaly. Morbidity and mortality most often results from infections acquired during severe neutropenia. The mechanism underlying the neutropenia is not well understood. Interestingly up to 40% of patients with T-cell LGL have rheumatoid arthritis. Although the cause of T-LGL leukemia and the events initiating the development of rheumatoid arthritis are now known, it has been hypothesized that there may be a common etiology underlying both diseases. Patients with NK-LGL possess clonally expanded LGL with a CD3-, CD4-, CD8-, CD16+ and CD56+ phenotype. In spite of aggressive treatment with multi-agent chemotherapy, 80% of these patients die within two months of diagnosis due to disseminated disease with multi-organ failure.

Cytotoxic T lymphocytes (CTL) are $CD8^+$ T cells activated in response to antigen. Such CTL can be categorized into naïve $CD8^+$ cells, terminally differentiated effector cells which are likely to undergo apoptosis, and a minor proportion of long-term $CD8^+$ memory cells. These memory cells proliferate in the presence of antigen (Butz *et al.*, 1998). Cell-mediated killing by cytotoxic T-lymphocytes is an important event to protect the host against viral infection and tumor cell proliferation (Crabtree *et al.*, 1994; Grakoui *et al.*, 1999). Cytotoxic T cells are loaded with granules containing various effector molecules that are capable of killing target cells. Upon contact with target cells, the cytotoxic cells release cytotoxic molecules vectorially into the target cells and destroy them. Once the antigen is cleared from the system, the majority of the cytotoxic T cells (terminally differentiated cells) die primarily through Fas-mediated apoptosis in order to maintain homeostasis (Nagata *et al.*, 1995; Callan *et al.*, 2000; Zimmerman *et al.*, 1996). In lymphoproliferative disorders such homeostasis is not maintained, resulting in the accumulation of a large number of lymphocytes. This may be due to defective apoptotic pathways in effector $CD8^+$ cells or due to the constant presence of antigen leading to a continuous proliferation of cells.

The T cell form of large granular lymphocyte (LGL) leukemia is a lymphoproliferative disorder often associated with autoimmune disease (Loughran, Jr., 1993; Lamy *et al.*, 1999). Several lines of research suggest that leukemic LGL are antigen activated CTL. Leukemic LGL display an activated cytotoxic T-cell phenotype (Loughran, Jr., 1993). Activation of leukemic LGL can be triggered through CD3 and/or CD16 pathways (Hoshino *et al.*, 1991; Loughran *et al.*, 1990). Leukemic LGL constitutively express perforin and Fas ligand which, besides NK cells, are found expressed only in T cells activated for killing (Oshimi *et al.*, 1990; Lamy *et al.*, 1998). A restricted T cells receptor repertoire has been

found in some studies of LGL leukemia, suggesting antigen selection (Zambello *et al.*, 1995; Kasten-Sportes *et al.*, 1994).

Brief Summary of the Invention

The subject invention concerns materials and methods for screening, diagnosis, and treatment of LGL leukemia and autoimmune disorders. A series of both known and novel genes sequences that are differentially expressed in LGL leukemia has been identified. One aspect of the invention provides for the use of these genes as molecular markers for LGL leukemia and also as novel therapeutic targets for the disease. Thus, another aspect of the invention pertains to therapeutic compositions directed to gene expression and gene products of differentially expressed genes in LGL. The invention also concerns methods for screening and identifying compositions that may be of therapeutic benefit to patients having LGL leukemia and/or autoimmune disorders. In addition, because a large fraction of patients with T-LGL leukemia also have rheumatoid arthritis, these differentially expressed genes also represent novel targets for the diagnosis, prevention or treatment of rheumatoid arthritis and other autoimmune diseases.

Brief Description of the Drawings

The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawings will be provided by the Patent Office upon request and payment of the necessary fee.

Figures 1A-1C show the cDNA microarray portions showing the expression of granzyme B/H, cathepsin W (Lymphopain) and perforin. cDNA microarray (UniGEM-V from Incyte Genomics) hybridized with the fluorescent probes prepared from mRNA isolated from PBMC of LGL leukemic patients (red) and from mRNA isolated from normal control (green). Images show the hybridization profile for an LGL patient and for the normal control. A color bar at the bottom shows the increased pattern of gene expression from left to right. **Figure 1A** shows a portion of the microarray showing the element F12 for granzyme B/H (indicated by the arrow, the cDNA fragment arrayed on microarray can hybridizes with both Granzyme B and H). **Figure 1B** shows a portion of the microarray showing the element D4 for Cathepsin W (indicated by the arrow). **Figure 1C** shows a portion of the microarray showing the element D2 for perforin (indicated by the arrow).

Figures 2A-2D show the Northern blot analysis of granzyme B/H, cathepsin W, perforin, and calpain. Northern blot analysis was performed with 10 μg of total RNA isolated from PBMC of leukemic patients and normal controls. Clones containing cDNA fragments were excised from the plasmids and used as probes. After hybridization with the corresponding gene probes, the Northern blots were stripped and reprobed with the housekeeping gene GAPDH and the bands were normalized using the ImageQuant program. LGL stands for LGL leukemia patients. N stands for normal. NA stands for normal. PBMC were activated by IL-2 and PHA as described in the Materials and Methods section. **Figure 2A** is the Northern blot showing the expression of granzyme B/H. **Figure 2B** is the Northern blot showing the expression of cathepsin W. **Figure 2C** is the Northern blot analysis showing the expression of perforin. **Figure 2D** is the Northern blot showing the expression of calpain.

Figures 3A-3F show RNase protection assays. RNase protection assay (RPA) was performed as described in the Materials and Methods section. LGL stands for leukemic patients. N stands for normal. NA stands for normal activated. Bands showing the mRNA expression were quantitated and normalized with the housekeeping gene, L32, using ImageQuant program and relative expression was given as arbitrary units for each sample. **Figure 3A** shows the hybridization profile for Granzyme B. **Figure 3B** shows the hybridization profile for Granzyme H. A probe set, hAPO4, was obtained containing Granzyme B, H. These probes are very specific and distinguish between granzyme B and H. **Figure 3C** shows the hybridization profile for Granzyme A. **Figure 3D** shows the hybridization profile of Granzyme K. A probe set, hAPO4, was obtained containing Granzyme A, K. **Figure 3E** shows the hybridization profile of perforin. **Figure 3F** shows the hybridization profile of caspase-8. Probe sets, hAPO4 and hAPO3e, were obtained containing perforin and caspase-8.

Figure 4 shows the expression of granzyme H (B) in leukemic LGL. Western blot analysis of proteins isolated from normal, activated PBMC and leukemic LGL. Antibodies raised against granzyme B was used in this blot. Since granzyme B cross-react with granzyme H, it is difficult to distinguish between granzyme B and H. N stands for normal PBMC. NA stands for normal activated PBMC. LGL stands for leukemic LGL.

Figures 5A-5C show protein array detection of cytokines from LGL leukemia and normal sera. Cytokine arrays were completed on 20 LGL leukemia and 6 normal sera pools

as described in the Materials and Methods section. Depicted above is a membrane from a representative normal sera sample (**Figure 5A**) and from LGL leukemia serum (**Figure 5B**). Each sample was subjected to array and subsequent densitometry analyses minimum of two times. In this particular example, these densitometry analyses showed that ENA, GRO, IL-1α, IL-6, IL-8, MCP-2, MCP-3, MCSF, MIP-1β, MIP-1α, RANTES, EGF, ANG, OSM, and TRO were overexpressed in the LGL samples. **Figure 5C** shows the layout of the cytokine antibodies deposited on the array. The names of the cytokines used in the array are: Epithelial cell-derived neutrophil attractant-78 (ENA)-78; granulocyte colony-stimulating factor (G-CSF); granulocyte monocyte-colony stimulating factor (GM-CSF); growth-regulated oncogene-alpha (GRO-α); interleukin- (IL-); interferon-gamma (INF-γ); monocyte chemoattractant protein- (MCP-); macrophage colony-stimulating factor (MCSF), macrophage-derived chemokine (MDC); monokine induced by interferon-gamma (MIG); macrophage inflammatory protein- (MIP-); regulated on activation, normal T expressed and secreted (RANTES); stem cell factor (SCF); stromal cell-derived factor-1 (SDF-1) alpha; thymus- and activation-regulated chemokine (TARC); transforming growth factor- (TGF-); tumor necrosis factor (TNF); epidermal growth factor (EGF), insulin-like growth factor I (IGF-I); angiotensin (Ang); oncostatin M (OSM); thrombopoietin (Tpo); vascular endothelial growth factor (VEGF); platelet-derived growth factor (PDGF); Positive (Pos); Negative (Neg).

Figures 6A and 6B show overexpression of RANTES in LGL leukemia. RNase protection assays (RPA) were performed as described in the Materials and Methods section (**Figure 6A**). LGL: LGL leukemia cells, N: normal cells, NA: activated normal cells. Bands showing the mRNA expression were quantified and normalized with the housekeeping gene L32. Relative expression was given as arbitrary units for each sample. 10 LGL leukemia samples and 5 normal samples were used for statistical analysis. T-tests were performed assuming unequal variances. The P value obtained for RANTES was $p < 0.01$. **Figure 6B** shows measurement of RANTES by ELISA. RANTES levels are displayed in ng/ml. Results represent the findings from two experiments. LGL: LGL leukemia sera, N: normal sera. *($p<0.001$): Determined by confidence interval testing and Z test to be significantly greater than normal levels.

Figures 7A and 7B show elevated MIP-1β expression in LGL leukemia. RPA data demonstrating the overexpression of MIP-1β is shown in **Figure 7A**. RPAs were performed as described in the Materials and Methods section. LGL: leukemic LGL, N: normal cells,

NA: activated normal cells. Bands corresponding to mRNA expression were quantified and normalized with the housekeeping gene, L32, using an ImageQuant program. Relative expression was given as arbitrary units for each sample. 10 leukemic samples and 5 normal samples were used for statistical analysis. T-tests were performed assuming unequal variances. The P value for MIP-1β was $p<0.001$. Serum MIP-1β levels were determined by ELISA as shown in **Figure 7B**. MIP-1β levels are depicted in pg/ml. Results represent the findings from two experiments. LGL: LGL leukemia patients sera, N: normal sera. *($p<0.001$): determined by confidence interval testing and Z test to be significantly greater than normal levels.

Figures 8A-8C show increased expression of MIP-1α, IL-1β, and IL-1Ra transcripts in LGL leukemia. RPAs were performed as described in the Materials and Methods section. Bands corresponding to mRNA expression were quantified and normalized with the housekeeping gene, L32, using ImageQuant. Relative expression was given as arbitrary units for each sample. LGL: leukemia cell LGL, N: normal cells, NA: activated normal cells. **Figure 8A** shows RPA for MIP-1α: 10 LGL leukemia samples and 5 normal samples were used for statistical analysis. T-tests were performed assuming unequal variances. The P value obtained for MIP-1α was $p<0.02$. **Figure 8B** shows RPA for IL-1β: 12 LGL leukemia samples and 4 normal samples were tested for statistical analysis. T-test analyses were performed assuming unequal variances. The P value obtained for IL-1β was $p<0.05$. **Figure 8C** shows RPA for IL-Ra: 12 LGL leukemia samples and 4 normal samples were processed for statistical analysis. T-tests were performed assuming unequal variances. The P value obtained for IL-1Ra mRNA was $p<0.001$.

Figures 9A-9C show elevated IL-10, IL-12p35, and IL-8 mRNA expression in LGL leukemia. RPAs were performed as described in the Materials and Methods section. Bands identified as IL-10 mRNA were quantified and normalized with the housekeeping gene, L32, using an ImageQuant program. LGL: leukemic LGL, N: normal PBMCs, NA: normal activated PBMCs. Relative expression was given as arbitrary units for each sample. **Figure 9A** shows RPA for IL-10: 12 LGL leukemia samples and 4 normal samples were analyzed. T-tests were performed assuming unequal variances. The P value obtained for IL-10 was $p<0.02$. **Figure 9B** shows RPA for IL-12p35: 12 LGL leukemia samples and 4 normal samples were analyzed. T-tests were performed assuming unequal variances. The P value obtained for IL-12p35 was $p<0.02$. **Figure 9C** shows RPA for IL-8: 10 LGL samples and 5

normal samples were used for statistical analysis. T-tests were performed assuming unequal variances. The P value obtained for IL-8 was $p<0.055$.

Figures 10A and 10B show elevated levels of IL-18 and IFNγ mRNA expression in LGL leukemia. RPAs were performed as described in the Materials and Methods section. Bands corresponding to IFNγ mRNA were quantified and normalized with the housekeeping gene, L32, using ImageQuant. Relative expression was given as arbitrary units for each sample. LGL: leukemic LGL, N: normal PBMCs, NA: normal activated PBMCs. **Figure 10A** shows RPA for IFNγ: 12 LGL leukemia samples and 4 normal samples were analyzed. T-tests were performed assuming unequal variances. The P value obtained for IFNγ was $p<0.02$. **Figure 10B** shows RPA for IL-18: 10 LGL leukemia samples and 5 normal samples were analyzed. The P value obtained for IL-18 was $p<0.01$.

Detailed Disclosure of the Invention

The subject invention concerns methods and materials for screening for, detecting, and diagnosing LGL leukemia and autoimmune disorders in a person or animal. Using a combination of microarray, Rnase protection assay and Northern Blot analysis, a series of both known and novel genes that are differentially expressed in LGL were identified. A list of genes that are differentially expressed in LGL leukemia are shown in Tables 1, 2, and 3. Table 1 identifies differentially expressed genes in LGL1 and LGL2. This data is based on Incyte Genomics and Affymetrix Chip FL 6800. Table 2 identifies genes that are upregulated in LGL1, LGL2, and LGL3/RA. This data is based on Affymetrix U 95. Table 3 identifies genes that are downregulated in LGL leukemia patients when compared to normal. This data is based on Affymetrix U 95. These genes can be used as biological markers for LGL leukemia. Differentially expressed genes identified in the present invention can also be used as therapeutic targets for the treatment or prevention of LGL leukemia and also rheumatoid arthritis and other autoimmune diseases. Several cytokines that are constitutively produced in LGL were also identified using Rnase protection assays, cytokine protein array screening, and ELISAs.

One embodiment of a method of the invention comprises obtaining a biological sample from a person or animal, and screening for upregulated expression of a gene or genes whose expression is upregulated in LGL and/or screening for downregulated expression of a gene or genes whose expression is downregulated in LGL. Quantitative or qualitative expression can be determined using any suitable method known in the art including, but not

limited to, reverse transcription–polymerase chain reaction (RT-PCR), cDNA or oligonucleotide microarray analysis, and Northern blot analysis. Methods for polymerase chain reaction (PCR) are known in the art and have been described in U.S. Patent Nos. 4,683,195; 4,683,202; and 4,800,159.

In one embodiment of the methods, RNA from a patient's cells is screened for changes in RNA expression of targeted genes as compared to the levels of expression observed for RNA expression of the same genes from a normal or non-LGL patient or compared to a control RNA. In one embodiment, genes encoding proteases, cytokines, and/or other molecules identified herein as differentially expressed in LGL are screened for upregulation of expression, which is indicative of LGL leukemia and/or an autoimmune disorder. In another embodiment, genes encoding protease inhibitors and/or other molecules are screened for downregulation, which is indicative of LGL leukemia and/or an autoimmune disorder. In a further embodiment, genes encoding proteases, cytokines, and/or other molecules are screened for upregulation of expression and genes encoding protease inhibitors and/or other molecules are screened for downregulation of expression. Genes whose expression is upregulated in LGL and which are contemplated within the scope of the invention include, but are not limited to, protease encoding genes, for example, serine proteases (granzymes A, B, H, and K), cysteine proteases (cathepsin C and W), calpain small subunit and caspase-8, and cytokine encoding genes, for example, RANTES, MIP-1alpha, MIP-1beta, IL-1 beta, IL-8, IL-1Ra, IFN-gamma, IL-18, IL-10, and IL-12 p35. Genes whose expression is downregulated in LGL and which are contemplated within the scope of the invention include, but are not limited to, protease inhibitor encoding genes, for example, cystatin C and A, α-1 antitrypsin, and metalloproteinase inhibitors. Any embodiment of the invention can also optionally include screening for upregulation of genes encoding perforins, A 20, phosphatase in activated cells (PAC-1) (Kothapalli *et al.*, 2003), NGK2 receptors, sphingosine-1-phosphate receptor (Kothapalli *et al.*, 2002b), and other genes whose expression is upregulated in LGL as shown in Tables 1 and 2. Any embodiment of the invention can also optionally include screening for downregulation of other genes whose expression is downregulated in LGL as shown in Tables 1 and 3.

In a further embodiment of the subject methods, a biological sample from a person or animal is obtained, and screened for expression of, or increased level of expression of, a protein that is encoded by a gene whose expression is upregulated in LGL and/or screening for lack of expression, or decreased level of expression of, a protein that is encoded by a gene

whose expression is downregulated in LGL. Quantitative or qualitative expression can be determined using any suitable method known in the art including, but not limited to ELISA assay, Western blot analysis, and protein array screening.

In one embodiment of the methods, protein from a patient's cells is screened for changes in levels of expression of protein of a targeted gene as compared to the levels of expression observed for protein of the same gene from a normal or non-LGL patient or compared to a control protein level. In one embodiment, proteases, cytokines, and/or other molecules identified herein as differentially expressed in LGL are screened for increased level of expression, which is indicative of LGL leukemia and/or an autoimmune disorder. In another embodiment, protease inhibitors and/or other molecules are screened for decreased level of expression, which is indicative of LGL leukemia and/or an autoimmune disorder. In a further embodiment, proteases, cytokines and/or other molecules are screened for increased level of expression and protease inhibitors and/or other molecules are screened for decreased level of expression. Proteins whose expression is increased in LGL and are contemplated within the scope of the invention include protease encoding genes, for example, serine proteases (granzymes A, B, H, and K), cysteine proteases (cathepsin C and W), calpain small subunit and caspase-8, and cytokine encoding genes, for example, RANTES, MIP-1alpha, MIP-1beta, IL-1 beta, IL-8, IL-1Ra, IFN-gamma, IL-18, IL-10, and IL-12 p35. Proteins whose expression is decreased in LGL and are contemplated within the scope of the invention include protease inhibitor encoding genes, for example, cystatin C and A, α-1 antitrypsin, and metalloproteinase inhibitors. Any embodiment of the invention can also optionally include screening for increased expression of perforins, A 20, phosphatase in activated cells (PAC-1), NGK2 receptors, and other proteins whose expression is increased in LGL as shown in Tables 1 and 2. Any embodiment of the invention can also optionally include screening for decreased expression of other proteins whose expression is decreased in LGL as shown in Tables 1 and 3.

One can compare expression results from a method of the present invention with a statistically significant expression value obtained from a reference group of normal patients and/or patients that have LGL leukemia in order to determine whether the test sample exhibits increased or decreased or unchanged levels of expression of a gene or gene product of the invention.

In one embodiment of the subject methods, the expression of at least five genes or gene products whose upregulation is associated with LGL is determined. In another

embodiment, the expression of at least ten genes or gene products whose upregulation is associated with LGL is determined. In a further embodiment, the expression of at least 15 genes or gene products whose upregulation is associated with LGL is determined. In still a further embodiment, the expression of at least 20, at least 25, at least 30, at least 35, or at least 40 or more genes or gene products whose upregulation is associated with LGL is determined.

In one embodiment of the subject methods, the expression of at least five genes or gene products whose downregulation is associated with LGL is determined. In another embodiment, the expression of at least ten genes or gene products whose downregulation is associated with LGL is determined. In a further embodiment, the expression of at least 15 genes or gene products whose downregulation is associated with LGL is determined. In still a further embodiment, the expression of at least 20, at least 25, at least 30, at least 35, or at least 40 or more genes or gene products whose downregulation is associated with LGL is determined.

The biological sample used in the methods and materials of the invention can be from any suitable biological tissue or fluid, including but not limited to bone marrow, lymph node, spleen, peripheral blood, lymph fluid, serous fluid, urine, saliva, and the like.

The subject invention also concerns kits comprising materials and compositions for use in screening for, detecting and diagnosing LGL or autoimmune disorders. The materials provide for detecting or determining expression of genes, and/or proteins encoded thereby, whose expression is differentially upregulated or downregulated in LGL as compared to expression levels in normal cells. In one embodiment, the screening materials comprise an array having one or more target gene or polynucleotide sequence whose expression is upregulated or downregulated in LGL. Nucleic acid samples can be obtained from a person or animal and the level of expression in the person or animal of the targeted gene or polynucleotide sequence provided on the array can be determined following hybridization of the sample with the array. In one embodiment, the array comprises one or more of the following target gene or polynucleotide sequences: granzymes A, B, H, and K; cathepsin C and W; calpain small subunit; caspase-8; cystatin C and A; α-1 antitrypsin; metalloproteinase inhibitor-8; perforins; A 20; PAC-1; NGK2 receptors; RANTES; MIP-1alpha; MIP-1beta; IL-1 beta; IL-8; IL-1Ra; IFN-gamma; IL-18; IL-10; IL-12 p35.

In another embodiment, a kit of the invention comprises oligonucleotide probes and PCR primers having sequences complementary to a sequence of a gene or polynucleotide

(sequences of which correspond to the sequences in the accession numbers and identification numbers provided herein) whose expression is differentially expressed in LGL. In another embodiment, a kit of the invention provides for RT-PCR of nucleic acid samples for detecting expression levels of a gene or polynucleotide whose expression is differentially expressed in LGL.

In another embodiment, a kit of the invention comprises an antibody or antibodies that bind to gene products that are differentially expressed in LGL. The antibodies can be provided on an array.

The materials and compositions of a kit of the invention can be provided in one or more separate containers.

The subject invention concerns methods for treating LGL leukemia or an autoimmune disorder comprising administering an effective amount of a composition that inhibits the expression of a gene or polynucleotide, or that inhibits or blocks biological activity of a protein encoded by the gene or polynucleotide, that is upregulated in LGL,. The subject invention also concerns methods for treating LGL leukemia or an autoimmune disorder comprising administering an effective amount of a composition that increases expression of a gene or polynucleotide, or that increases expression or level of a protein encoded by the gene or polynucleotide, that is downregulated in LGL,.

Genes and polynucleotides whose expression is increased in LGL and can be the targets for inhibition in the subject methods include, but are not limited to, protease encoding genes, for example, serine proteases (granzymes A, B, H, and K), cysteine proteases (cathepsin C and W), calpain small subunit and caspase-8, and cytokine encoding genes, for example, RANTES, MIP-1alpha, MIP-1beta, IL-1 beta, IL-8, IL-1Ra, IFN-gamma, IL-18, IL-10, and IL-12 p35. Genes and polynucleotides whose expression is decreased in LGL and can be the targets for increased expression include, but are not limited to, protease inhibitor encoding genes, for example, cystatin C and A, α-1 antitrypsin, and metalloproteinase inhibitors. Any embodiment of the methods of the invention can also optionally include inhibiting expression of genes or polynucleotides that encode perforins, A 20, phosphatase in activated cells (PAC-1), NGK2 receptors, and other proteins whose expression is increased in LGL as shown in Tables 1 and 2, and/or increasing expression of other genes or polynucleotides whose expression is decreased in LGL as shown in Tables 1 and 3. One embodiment of the subject method comprises upregulating or increasing expression of genes

encoding protease inhibitors or contacting an LGL with a protease inhibitor whose expression is downregulated in LGL.

Means for inhibiting expression of a specific targeted gene are known in the art and include antisense nucleic acid inhibition and RNA interference (RNAi). Means for inhibiting or blocking biological activity of a protein are also known in the art and include, for example, antibodies that specifically bind to a protein and block biological activity of the protein or that bind to the cellular receptor for the protein and prevent or inhibit binding of the protein to the receptor. Peptides can also be used that bind to a protein or receptor and block biological activity.

Polynucleotides that provide for transcribed sequences that are at least partially complementary to the transcribed sequence of a gene whose expression is upregulated in LGL, such as a gene encoding a protease enzyme or a cytokine, are also contemplated within the scope of the present invention. Such polynucleotides are referred to herein as antisense polynucleotides and the sequences are antisense sequences. Transcription of the antisense sequence results in production of RNA which is at least partially complementary to RNA transcribed from a gene. In one embodiment, the polynucleotide comprises a nucleotide sequence that is antisense to a sequence of a gene having a nucleotide sequence disclosed in an accession number or identification number herein. The polynucleotide does not have to be identical in sequence to or the same length as the endogenous gene sequence. The polynucleotide used for antisense inhibition can be shorter in length than the full-length gene sequence. For example, a polynucleotide can be used that corresponds to the 5'-end or the 3'-end of the endogenous gene.

The polynucleotide sequence that is complementary to a sequence of an mRNA of a target gene whose expression is to be inhibited is selected to be of sufficient length to bind to the mRNA and inhibit expression of the enzyme. The sequence is preferably between 10 and 5000 nucleotides in length. More preferably, the sequence is between 20 and 2000 nucleotides in length. Most preferably, the sequence is between 50 and 1000 nucleotides in length. The sequence transcribed from the antisense polynucleotide may be complementary to any sequence of the RNA transcribed from the target gene, including the 5' non-coding sequence, 3' non-coding sequence, introns, the coding sequence, or any portion thereof.

RNA interference (RNAi) can also be used to suppress or inhibit expression of an endogenous gene (McManus and Sharp, 2002; published U.S. patent application No. US2003/0190654 A1; published international application No. PCT/GB00/04404). In one

embodiment of RNAi, short interfering double-stranded RNAs (siRNA) of about 20-25 nucleotides, and more typically of 21-23 nucleotides, in size and complementary to strands of the gene to be silenced are provided in a cell. For example, siRNAs that have 20-25 nucleotide, or 21-23 nucleotide, strands complementary to a nucleotide sequence of a gene whose expression that is upregulated in LGL are contemplated within the scope of the present invention. A vector that has a nucleotide sequence that when transcribed in a cell produces one or more separate siRNA strands that can then form the duplex form of the siRNA can be introduced into a targeted LGL cell.

In another embodiment of RNAi, a short hairpin RNA molecule (shRNA) is expressed in a cell. The shRNA, consisting of short inverted repeats separated by a small loop sequence, are expressed from a suitable vector. One inverted repeat is complementary to the gene target. The shRNA is then processed into an siRNA which suppresses expression of the gene to be silenced. A vector that has a nucleotide sequence that when transcribed in the cell produces one or more separate shRNA strands that can then form a hairpin can be introduced into a targeted LGL cell.

In addition to humans, animals can also be treated using the subject methods. Animals contemplated with the scope of the invention include, but are not limited to, mammals such as primates (monkey, chimpanzee, *etc.*), dog, cat, cow, pig, or horse, or other animals that have LGL leukemia or an autoimmune disorder.

The subject invention also concerns compositions for treating or preventing large granular lymphocyte (LGL) leukemia or an autoimmune disorder in a person or animal, wherein the composition comprises a means for inhibiting expression of a gene or polynucleotide, or inhibiting or blocking biological activity of a protein encoded by a gene or polynucleotide, whose expression is upregulated in LGL. In one embodiment, the composition comprises an antisense polynucleotide whose transcribed sequence is at least partially complementary to the transcribed sequence of a gene whose expression is upregulated in LGL, wherein expression of said gene is inhibited or blocked by expression of said antisense polynucleotide. In a further embodiment, the gene is granzymes A, B, H, or K; cathepsin C or W; calpain small subunit; caspase-8; perforins; A 20; PAC-1; NGK2 receptors; RANTES; MIP-1alpha; MIP-1beta; IL-1 beta; IL-8; IL-1Ra; IFN-gamma; IL-18; IL-10; or IL-12 p35, or one of the genes listed in Tables 1 and 2 whose expression is upregulated in LGL.

In another embodiment, a composition of the invention comprises an RNA that interferes with expression of a gene or polynucleotide whose expression is upregulated in LGL. In one embodiment, an RNA interfering molecule of the invention inhibits expression of one of the following genes: granzymes A, B, H, or K; cathepsin C or W; calpain small subunit; caspase-8; perforins; A 20; PAC-1; NGK2 receptors; RANTES; MIP-1alpha; MIP-1beta; IL-1 beta; IL-8; IL-1Ra; IFN-gamma; IL-18; IL-10; or IL-12 p35, or one of the genes listed in Tables 1 and 2 whose expression is upregulated in LGL.. The RNA interfering molecule can be provided in the form of an siRNA.

In still another embodiment, a composition of the invention can comprise an antibody, or an antigen binding fragment thereof, that specifically binds to a protein encoded by a gene or polynucleotide whose expression is upregulated in LGL and blocks biological activity of the protein; an antibody, or an antigen binding fragment thereof, that specifically binds to a receptor for the protein and prevents or inhibits binding of the protein to the receptor; a peptide that binds to the protein or thereceptor and block biological activity of the protein or the receptor; or a combination of any of antibody or peptide.

The subject invention also concerns compositions for treating or preventing large granular lymphocyte (LGL) leukemia or an autoimmune disorder in a person or animal, wherein the composition comprises a means for increasing expression or levels of a protein encoded by a gene or polynucleotide whose expression is downregulated in LGL, such as the protease inhibitors cystatin C and A, α-1 antitrypsin, and metalloproteinase inhibitors.

In one embodiment, methods and compositions for treatment of LGL and/or autoimmune disorders can include inhibitors of those proteases whose expression is upregulated in LGL as described herein.

Therapeutic compositions of the invention can be delivered to a cell by direct contact with the cell or via a carrier means. Carrier means for delivering compositions to cells are known in the art and include encapsulating the composition in a liposome moiety, and attaching a oligonucleotide, peptide, etc. to a protein or nucleic acid that is targeted for delivery to the target cell. Published U.S. Patent Application Nos. 2003/0032594 and 2002/0120100 disclose amino acid sequences that can be coupled to another composition and that allows the composition to be translocated across biological membranes. Published U.S. Patent Application No. 2002/0035243 also describes compositions for transporting biological moieties across cell membranes for intracellular delivery.

For the treatment of oncological disorders, the therapeutic compositions of this invention can be administered to a patient in need of treatment in combination with other antitumor substances, with radiation therapy, and the like. These other substances or radiation treatments may be given at the same or different times as the therapeutic compositions of this invention. For example, therapeutic compositions of the present invention can be used in combination with mitotic inhibitors such as taxol or vinblastine, alkylating agents such as cyclophosamide or ifosfamide, antimetabolites such as 5-fluorouracil or hydroxyurea, DNA intercalators such as adriamycin or bleomycin, topoisomerase inhibitors such as etoposide or camptothecin, antiangiogenic agents such as angiostatin, antiestrogens such as tamoxifen, and/or other anti-cancer drugs or antibodies.

Therapeutic application of the therapeutic compositions, and compositions containing them, can be accomplished by any suitable therapeutic method and technique presently or prospectively known to those skilled in the art. Therapeutic compositions can be administered by any suitable route known in the art including, for example, oral, nasal, rectal, and parenteral routes of administration. As used herein, the term parenteral includes subcutaneous, intravenous, intramuscular, and intrasternal administration, such as by injection. Administration of therapeutic compositions of the invention can be continuous or at distinct intervals as can be readily determined by a person skilled in the art.

Therapeutic compositions of the subject invention can be formulated according to known methods for preparing pharmaceutically useful compositions. Formulations are described in detail in a number of sources which are well known and readily available to those skilled in the art. For example, *Remington's Pharmaceutical Science* by E.W. Martin describes formulations which can be used in connection with the subject invention. In general, the compositions of the subject invention will be formulated such that an effective amount of the bioactive composition is combined with a suitable carrier in order to facilitate effective administration of the composition. The compositions used in the present methods can also be in a variety of forms. These include, for example, solid, semi-solid, and liquid dosage forms, such as tablets, pills, powders, liquid solutions or suspension, suppositories, injectable and infusible solutions, and sprays. The preferred form depends on the intended mode of administration and therapeutic application. The compositions also preferably include conventional pharmaceutically acceptable carriers and diluents which are known to those skilled in the art. Examples of carriers or diluents for use with therapeutic compositions include ethanol, dimethyl sulfoxide, glycerol, alumina, starch, and equivalent

carriers and diluents. To provide for the administration of such dosages for the desired therapeutic treatment, pharmaceutical compositions of the invention will advantageously comprise between about 0.1% and 99%, and especially, 1 and 15% by weight of the total of one or more of a therapeutic composition of the invention based on the weight of the total composition including carrier or diluent.

Therapeutic compositions of the subject invention can also be administered utilizing liposome technology, slow release capsules, implantable pumps, and biodegradable containers. These delivery methods can, advantageously, provide a uniform dosage over an extended period of time.

The subject invention also concerns a packaged dosage formulation comprising in one or more containers at least one therapeutic compound of the subject invention formulated in a pharmaceutically acceptable dosage.

The subject invention also concerns methods for screening for compounds useful in treating or preventing LGL leukemia. In one embodiment, an LGL cell is contacted with a test compound and nucleic acid isolated from the cell and screened for: 1) inhibition of those gene sequences that are upregulated in LGL, or 2) increased expression of those gene sequences that are downregulated in LGL, or 3) both screening for inhibition of those gene sequences that are upregulated in LGL and screening for increased expression of those gene sequences that are downregulated in LGL are performed. Those gene sequences that are typically upregulated in LGL and that can be used in the subject methods include, but are not limited to, genes encoding granzymes A, B, H, and K; cathepsin C and W; calpain small subunit; caspase-8; perforins; A 20; PAC-1; NGK2 receptors; RANTES; MIP-1alpha; MIP-1beta; IL-1 beta; IL-8; IL-1Ra; IFN-gamma; IL-18; IL-10; IL-12 p35. Those gene sequences that are typically downregulated in LGL and that can be used in the subject method include, but are not limited to, genes encoding cystatin C and A; α-1 antitrypsin; metalloproteinase inhibitors. Alternatively, one can screen the cells contacted with the test compound for increased or decreased production or levels of proteins encoded by genes or polynucleotides that are differentially expressed in LGL, such as granzymes A, B, H, and K; cathepsin C and W; calpain small subunit; caspase-8; perforins; A 20; PAC-1; NGK2 receptors; RANTES; MIP-1alpha; MIP-1beta; IL-1 beta; IL-8; IL-1Ra; IFN-gamma; IL-18; IL-10; IL-12 p35; cystatin C and A; α-1 antitrypsin; and metalloproteinase inhibitors. Compounds identified as inhibiting expression of upregulated sequences and/or increasing expression of downregulated sequences are potential candidates for use in treating LGL.

The subject invention also concerns methods for screening for compounds useful in treating or preventing autoimmune disorders associated with LGL. In one embodiment, a cell is contacted with a test compound and nucleic acid isolated from the cell and screened for: 1) inhibition of those gene sequences that are upregulated in LGL, or 2) increased expression of those gene sequences that are downregulated in LGL, or 3) both screening for inhibition of those gene sequences that are upregulated in LGL and screening for increased expression of those gene sequences that are downregulated in LGL are performed. Those gene sequences that are typically upregulated in LGL and that can be used in the subject methods include, but are not limited to, genes encoding granzymes A, B, H, and K; cathepsin C and W; calpain small subunit; caspase-8; perforins; A 20; PAC-1; NGK2 receptors; RANTES; MIP-1alpha; MIP-1beta; IL-1 beta; IL-8; IL-1Ra; IFN-gamma; IL-18; IL-10; IL-12 p35. Those gene sequences that are typically downregulated in LGL and that can be used in the subject method include, but are not limited to, genes encoding cystatin C and A; α-1 antitrypsin; metalloproteinase inhibitor. Compounds identified as inhibiting expression of upregulated sequences and/or increasing expression of downregulated sequences are potential candidates for use in treating autoimmune disorders.

The subject invention also concerns variants of the genes and polynucleotides contemplated within the scope of the present invention. Variant sequences include those sequences wherein one or more nucleotides of the sequence have been substituted, deleted, and/or inserted. The nucleotides that can be substituted for natural nucleotides of DNA have a base moiety that can include, but is not limited to, inosine, 5-fluorouracil, 5-bromouracil, hypoxanthine, 1-methylguanine, 5-methylcytosine, and tritylated bases. The sugar moiety of the nucleotide in a sequence can also be modified and includes, but is not limited to, arabinose, xylulose, and hexose. In addition, the adenine, cytosine, guanine, thymine, and uracil bases of the nucleotides can be modified with acetyl, methyl, and/or thio groups. Sequences containing nucleotide substitutions, deletions, and/or insertions can be prepared and tested using standard techniques known in the art.

Genes and polynucleotides contemplated within the scope of the subject invention can also be defined in terms of more particular identity and/or similarity ranges with those sequences of the invention specifically exemplified herein. The sequence identity will typically be greater than 60%, preferably greater than 75%, more preferably greater than 80%, even more preferably greater than 90%, and can be greater than 95%. The identity and/or similarity of a sequence can be 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62,

63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% as compared to a sequence exemplified herein. Unless otherwise specified, as used herein percent sequence identity and/or similarity of two sequences can be determined using the algorithm of Karlin and Altschul (1990), modified as in Karlin and Altschul (1993). Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul *et al.* (1990). BLAST searches can be performed with the NBLAST program, score = 100, wordlength = 12, to obtain sequences with the desired percent sequence identity. To obtain gapped alignments for comparison purposes, Gapped BLAST can be used as described in Altschul *et al.* (1997). When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (NBLAST and XBLAST) can be used. *See* NCBI/NIH website.

The subject invention also contemplates those polynucleotide molecules having sequences which are sufficiently homologous with the polynucleotide sequences exemplified herein so as to permit hybridization with that sequence under standard stringent conditions and standard methods (Maniatis *et al.*, 1982). As used herein, "stringent" conditions for hybridization refers to conditions wherein hybridization is typically carried out overnight at 20-25 C below the melting temperature (Tm) of the DNA hybrid in 6x SSPE, 5x Denhardt's solution, 0.1% SDS, 0.1 mg/ml denatured DNA. The melting temperature, Tm, is described by the following formula (Beltz *et al.*, 1983):

Tm=81.5 C+16.6 Log[Na+]+0.41(%G+C)-0.61(% formamide)-600/length of duplex in base pairs.

Washes are typically carried out as follows:

(1) Twice at room temperature for 15 minutes in 1x SSPE, 0.1% SDS (low stringency wash).

(2) Once at Tm-20 C for 15 minutes in 0.2x SSPE, 0.1% SDS (moderate stringency wash).

As used herein, the terms "nucleic acid" and "polynucleotide" refer to a deoxyribonucleotide, ribonucleotide, or a mixed deoxyribonucleotide and ribonucleotide polymer in either single- or double-stranded form, and unless otherwise limited, would encompass known analogs of natural nucleotides that can function in a similar manner as naturally-occurring nucleotides. The polynucleotide sequences include the DNA strand sequence that is transcribed into RNA and the strand sequence that is complementary to the DNA strand that is transcribed. The polynucleotide sequences also include both full-length

sequences as well as shorter sequences derived from the full-length sequences. Allelic variations of the sequences also fall within the scope of the subject invention. The polynucleotide sequence includes both the sense and antisense strands either as individual strands or in the duplex.

Nucleotide and amino acid sequences of genes, and proteins encoded thereby, that are contemplated within the scope of the present invention include those sequences provided in publicly accessible sequence databases such as Genbank and which are identified herein (such as in Tables 1, 2, and 3) by accession number or identification number, including those incorporated by reference.

All patents, patent applications, provisional applications, and publications referred to or cited herein are incorporated by reference in their entirety, including all sequences (including those identified by database accession number), figures and tables, to the extent they are not inconsistent with the explicit teachings of this specification.

Materials and Methods

Isolation of PBMC and RNA.

PBMC were isolated from whole blood using Ficoll-Hypaque density gradient centrifugation. These cells were suspended in Trizol reagent (GIBCO-BRL, Rockville, MD) and total RNA was isolated immediately according to the manufacturer's instructions. Poly A^+ RNA was isolated from total RNA by using Oligo-Tex mini mRNA kit (Qiagen, Valencia, CA) according to the manufacturer's recommendations. All patients selected had T cell form of LGL leukemia.

Activation of PBMC.

Normal PBMC were cultured *in vitro* and activated using PHA (Sigma Chemical Co., St. Louis, MO) (1 μg/ml, 2 days) and Interleukin-2 (IL-2) (100 U/ml, 10 days), then total RNA was isolated.

cDNA Microarray.

Microarray probing and analysis was done by Incyte Genomics. Briefly, one μg of Poly $(A)^+$ RNA isolated from PBMC of an LGL leukemia patient and a healthy individual

was reverse transcribed to generate Cy3 and Cy5 fluorescent labeled cDNA probes. cDNA probes were competitively hybridized to a human UniGEM-V cDNA microarray containing 7075 immobilized cDNA fragments (4107 for known genes and 2968 ESTs). Microarrays were scanned in both Cy3 and Cy5 channels with Axon GenePix (Foster City) with a 10 μm resolution. Incyte GEMtools software (Incyte Pharmaceuticals, Inc., Palo Alto, CA) was used for image analysis. The elements were determined by gridding and region detection algorithm. The area surrounding each element image was used to calculate a local background and was subtracted from the total element signal. Background subtracted element signals were used to calculate the Cy3:Cy5 ratio. The average of the resulting total Cy3 and Cy5 signal provided a ratio that was used to balance or normalize the signals. P1 and P2 signals were the intensity reading obtained by the scanner for Cy3 and Cy5 channels. The balanced differential expression was calculated using the ratio between the P1 signal (intensity reading for probe 1) and the balanced P2 signal (intensity reading for probe 2 adjusted using the balanced coefficient).

Microarray Analysis using Oligonucleotide Probe Arrays.

The HuGeneFL (contains 6800 genes) microarray chip obtained from Affymetrix (Santa Clara, CA) was used. Briefly total RNA isolated from normal PBMC of normal, normal sorted $CD8^+$ T cells and PBMC from two different LGL leukemia patients (designated herein as LGL 1 and LGL 2, respectively) were DNase treated and purified with a Qiagen kit. Approximately 10 μg of purified RNA was used to prepare double stranded cDNA (superscript GIBCO/BRL) using a T7 (dT)24 primer containing a T7 RNA polymerase promoter binding site. Biotinylated complementary RNA was prepared from 10 μg of cDNA and then fragmented to approximately 50 to 100 nucleotides. *In vitro* transcribed transcripts were hybridized to the HuGeneFL microarray chip for 16 h at 45 °C with constant rotation at 60 rpm. Chips were washed and stained by using Affymetrix fluidics station. Fluorescence intensity was measured for each chip and normalized to the fluorescence intensity for the entire chip.

Verification of the Clones.

GEM cDNA clones (each clone was supplied as a bacterial stab) were purchased from Incyte Genomics and streaked on to LB/agar plates containing the appropriate antibiotic.

Individual colonies were picked and cultured in LB medium. Plasmid DNA was isolated and sequenced in order to verify the sequence identity.

Northern Blot Analysis.

Northern Blotting was done as described in the standard protocols (Sambrook, 1989). Briefly 10 μg of total RNA of each sample was denatured at 65 °C in RNA loading buffer, electrophoresed in a 1% agarose gel containing 2.2 M formaldehyde, then blotted onto a Nytran membrane (Schleicher & Schuell, Inc., Keene, N.H). The RNA was fixed to the membrane by UV cross-linking. cDNA probes were labeled with [^{32}P] and purified by Nick columns (Amersham Pharmacia Biotech AB, Piscataway, NJ). Hybridization and washings of the blots were performed as described by Engler-Blum *et al.* (1993). The blots were exposed to X-ray films and after developing the film, the bands were quantitated by using the ImageQuant program and normalized with the housekeeping gene GAPDH.

RNase Protection Assay (RPA) for Proteases and Protease Inhibitors.

RPA was performed using the RNA isolated from leukemic LGL, normal PBMC and normal PBMC activated by IL-2 and PHA. Five μg of total RNA was hybridized to the *in vitro* transcribed hAPO4 and hAPO3c probe sets (PharMingen, SanDiego, CA), the RPA assay was performed according to the manufacture's protocol. After assay, the samples were resolved on a 5% polyacrylamide gel. The gel was dried and exposed to X-ray film. After developing the film, the bands were quantitated by using the ImageQuant program and normalized with the housekeeping gene, L32.

Western Immunoblotting.

Cells were lysed in a buffer containing 50 mM Tris-HCl (pH 7.6); 5mM EDTA; 150 mM NaCl; 0.5 % NP-40; 0.5% Triton X-100 containing 1μg/ml leupeptin, aprotinin and antipain; 1 mM sodiumorthovanadate; and 0.5 mM PMSF (all reagents were obtained from Sigma Chemical Co. St. Louis, MO) 25 μg of total protein from each sample was subjected to 10% SDS-PAGE. Then the proteins were transferred to a membrane and Western blotting was performed by using the monoclonal antibody for granzyme B (2C5, Santa Cruz Biotechnology, Santa Cruz, CA) and the ECL technique as recommended by the manufacturer (Amersham Pharmacia Biotech, Piscataway, NJ).

RNase Protection Assay (RPA) for Cytokines.

RPA was performed using RNA isolated from leukemic LGL, normal PBMCs and normal PBMCs activated by IL-2 and PHA. Five μg of total RNA was hybridized to *in vitro* transcribed cytokine multi-probe sets (RiboQuant, BD Biosciences, San Jose, CA) and the RPA assay was performed according to the manufacturer's protocol. The samples were resolved on a 5% polyacrylamide gel. The gel was dried and exposed to X-ray film. After developing the film, the bands were quantified by using the ImageQuant program (Molecular Dynamics, Sunnyvale, CA) and normalized against the housekeeping gene, L32.

Cytokine Protein Array Screening.

LGL leukemia sera were screened for relative cytokine levels by cytokine protein arrays, following the kit manufacturer's directions (RayBiotech, Inc., Norcross, GA). Twenty LGL leukemia sera and six sets of pooled normal sera (12 donors for test) were tested. Each protein array membrane contained a grid of capture antibodies specific for 43 different human cytokines. Briefly, membranes were blocked, and then incubated with 10 fold-diluted sera for 2 hours. After washing, the membrane-bound serum components were reacted with a biotin-conjugated anti-cytokine antibody cocktail. After the non-binding conjugates were removed, the membranes were incubated with HRP-conjugated strepavidin, and then washed a final time. HRP-biotin conjugated complexes indicating the presence of human cytokines was visualized by ECL reactions on film. A two-step process was used to determine relative expression. First, densitometry analysis was completed on individual membranes, which contained positive and negative controls. Then, the densitometry data for each LGL leukemia sample was compared to the corresponding data for normal sera and an expression ratio was derived. The significance of fold differences were determined by the use of confidence interval testing derived from the densitometry results of each experiment.

Cytokine ELISAs.

Cytokines were selected for quantification based on RPA and/or protein array results. In general, ELISAs were performed for all cytokines and chemokines with increased levels of mRNA expression, unless protein array blot identified no differential protein expression for a particular cytokine/chemokine. Interleukin-1β (IL-1 β) and interleukin-8 (IL-8) were analyzed with OptEIA sets (PharMingen, San Diego, CA), interleukin-1 receptor antagonist

(IL-1Ra) and IL-18, were analyzed with Quantikine kits (R&D Systems, Minneapolis, MN) and all others were analyzed with kits or antibody pairs from Pierce Endogen. Additional testing for serum IL-1β was completed using the R&D Systems IL-1β Quantikine kit. For ELISAs, 27 LGL leukemia sera, 13 normal sera representing the age and gender distribution of LGL leukemia (purchased from Florida Blood Services, St. Petersburg, FL) plus pooled sera from an additional 12 normal donors (Sigma) were tested. All analyses were performed twice with the exception of IL-1β analyses, which were performed in quadruplicate. Manufacturer's instructions were followed for each cytokine tested.

Following are examples which illustrate procedures for practicing the invention. These examples should not be construed as limiting. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted.

Example 1—Screening for Differential Expression of Genes in LGL

Overexpression of a variety cytotoxic genes was observed in leukemic LGL utilizing cDNA microarray from Incyte Genomics (Figure 1 and Table 4). To verify the identity of these overexpressed genes, clones containing cDNA fragments of the selected genes were obtained from Incyte Genomics and confirmed by sequencing. Northern blots were then performed to confirm these results in samples from other LGL leukemia patients. For this analysis, we used the cDNA fragments (for the majority of the genes mentioned in the tables) obtained from the clones as probes (Incyte Genomics). All leukemic LGL showed constitutive expression of granzyme B and H, and cathepsin W (Figures 2A and 2B), whereas as a majority of patients showed overexpression of perforin (Figure 2C). A gene coding for calpain small polypeptide was also expressed in the majority of the leukemic LGL (Figure 2C). In addition to these cytotoxic genes, other genes were identified which were differentially expressed when comparing a sample from an LGL leukemia patient to a sample from a normal individual. Approximately 80 genes appeared upregulated and 12 downregulated in the cDNA microarray.

An Affymetrix chip was also used to identify differentially expressed genes in leukemic LGL. In these experiments, the expression of different genes was compared with normal PBMC, purified normal $CD8^+$ cells and leukemic LGL from two (2) patients. This analysis also showed the overexpression of genes coding for granzyme A, H, B, K and perforin. In addition, upregulation of cathepsin C (Table 5) was observed.

Protease inhibitors such as cystatin C, cystatin A, α-1 antitrypsin and metalloproteinase inhibitor were downregulated in leukemic LGL when compared to normal PBMC. In $CD8^+$ cells, these inhibitors were drastically downregulated when compared to both normal PBMC and leukemic LGL (Table 6). Because of a high degree of sequence similarity, it was not possible to distinguish granzyme B from granzyme H in microarrays and in Northern blots. Therefore, an RPA was performed using specific probes for granzyme B and H. The majority of samples from the LGL leukemia patients constitutively overexpressed both granzyme B and H (Figures 3A and 3B). Granzyme B was also upregulated in activated PBMC, whereas such upregulation was not observed with granzyme H. Granzyme A and K were also overexpressed in the majority of the patient's samples (Figures 3C and 3D). RPA also confirmed the upregulation of perforin and caspase-8 in the majority of LGL patients (Figures 3E and 3F). Normal PBMC express low levels of caspase-8, but upon activation of PBMC with IL-2 and PHA, the message levels of caspase-8 were further reduced and in some cases hardly detectable. In Western Blot experiments, overexpression of granzymes in leukemic LGL (Figure 4) was observed, although the antibody used in the experiment did not distinguish between granzyme B and granzyme H.

Example 2—CC and CXC Chemokine Expression: LGL Leukemia Samples Constitutively Express High Levels of RANTES, MIP-1β and IL-8

Protein arrays for 20 LGL leukemia sera and 6 sets of pooled normal sera were completed in duplicate. The most commonly elevated cytokines belonged to the CC chemokine family including RANTES, MIP-1β and IL-8 (Figure 5). Significant overexpression of RANTES (Figure 6A), MIP-1β transcripts (Figure 7A) and macrophage inflammatory protein-1α (MIP-1α) (Figure 8A) in leukemic LGL samples was observed. Elevated levels of IL-8 mRNA were found in some samples from patients with LGL leukemia (Figure 9C) and as a group achieved borderline significance ($P<0.055$). ELISA data further confirmed the elevated expression of RANTES, MIP-1β, and IL-8 proteins in LGL leukemia sera (Figures 6B and 7B and Table 7). While the mean RANTES levels for normal sera (N) as detected by the ELISA reagents was approximately 3ng/ml, RANTES levels in patient sera (LGL) ranged from 14 ng/ml to 20 ng/ml with a mean level of 17 ng/ml. ELISA testing revealed that MIP-1β secretion was significantly elevated in 16 of the 27 LGL leukemia sera (Figure 7B). Sera from LGL leukemia patients had significantly elevated IL-8

levels compared to normal sera due to the greatly increased amounts of IL-8 in 11 of the 27 sera tested (Table 7). In contrast to these findings, ELISA analysis for MIP-1α could not validate the RPA analysis showing increased levels of MIP-1α transcripts in each of 10 LGL leukemia patient samples. Of interest, densitometry analyses of the protein arrays had revealed that 6 of 20 LGL leukemia sera contained significantly elevated levels of MIP-1α. The ELISA results utilizing a larger number (27) of LGL leukemia samples showed that sera from 5 patients demonstrated significantly elevated amounts of this chemokine. Thus, the mean MIP-1α levels were not increased in sera from LGL leukemia patients compared to normal control sera (Table 7).

Example 3—Increased Levels of Other Cytokines in LGL Leukemia (IL-18, IL-Ra)

Levels of expression of a large number of cytokine gene transcripts were found not to be elevated in LGL leukemia samples by RPA include lymphotactin (Ltn), monocyte chemoattractant protein-1 (MCP-1), interleukin-1α (IL-1α), interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-6 (IL-6), interleukin-9 (IL-9), interleukin-14 (IL-14), interleukin-15 (IL-15) and tumor necrosis factor -α (TNF-α) (not shown). In contrast RPA results showed significantly increased levels of IL-1β, IL-1Ra, interleukin-10 (IL-10), interleukin-12 (IL-12), IL-18, interferon gamma (INF-γ) gene transcripts in these patient samples (Figures 8A-8C, Figures 9A-9C, and Figures 10A-10B). ELISA testing was then performed for each of these proteins, except for IL-10 and IL-12 as protein array testing did not detect increased levels of these proteins in LGL sera. Of note protein array testing showed overexpression of IL-1β in only 4 of 20 LGL leukemia samples. However, IL-1β ELISA testing was performed since a previous report utilizing both microarray and ELISA had suggested increased levels of this cytokine in a small group of patients with LGL leukemia. Although the IL-1β transcripts were elevated, the IL-1β protein levels in the LGL leukemia sera were not different than levels seen in normal sera.

Levels of IL-18, IL-1Ra, IFN-γ, and TNF-α were elevated in LGL leukemia patient samples to varying extents (Table 7) was demonstrated. Mean levels of IL-18 and IL-1Ra were significantly higher in LGL leukemia sera than normal sera. Although mean levels of INF-γ and TNF-α were not elevated, sera from 11 and 13 patients respectively did show increased levels of these cytokines.

Example 4—Other Protein Array Results

Many other growth factors or chemokines/lymphokines, not tested by RPA, were not differentially expressed when comparing results of twenty LGL leukemia sera to six sets of pooled normal sera utilizing the protein array. Such proteins included epithelial cell-derived neutrophil attractant-78 (ENA-78), granulocyte colony-stimulating factor (G-CSF), granulocyte monocyte-colony stimulating factor (GM-CSF), growth-regulated oncogene (GRO), growth-regulated oncogene-alpha (GRO alpha), IL-2, interleukin-3 (IL-3), interleukin-7 (IL-7), interleukin-13 (IL-13), monocyte chemoattractant protein-2 (MCP-2), monocyte chemoattractant protein-3 (MCP-3), macrophage colony-stimulating factor (MCSF), macrophage-derived chemokine (MDC), monokine induced by interferon-gamma (MIG), stem cell factor-1 (SCF-1), stromal cell-derived factor-1 (SDF-1), thymus- and activation-regulated chemokine (TARC), tumor growth factor-beta (TGF-β) epidermal growth factor (EGF), insulin-like growth factor 1 (IGF-1) and thrombopoitin (TPO). There was a suggestion that there might be elevated levels of endothelial or blood vessel growth factors as evidenced by increased angiotensin (ANG), vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) expression in at least in five of 20 LGL leukemia sera. Similar results were also found for leptin-I-309 and oncostatin M (OSM).

It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application.

Table 1. Differentially expressed genes in LGL1 and LGL2. This data is based on Incyte Genomics and Affymetrix Chip FL 6800

	Incyte Genomics		Affymetrix	
Gene Name	BDE (p1/p2)	GenBankID	Fold Change (LGL1/LGL2)	GenBank ID If different from Incyte Genomics
Upregulated Genes				
Proteolytic enzymes				
Granzyme H precursor	6.3 (3332/533)	M57888 NM033423 BC027974	(1.5/1.8) (21.8/10.8)	M37245 M28879
Lymphopain (Cathepsin W)	5.4 (3578/658)	AF013661 NM001335 BC035637 BC048255	———	
Perforin	3.8 (1549/413)	L40557 BC063043 X13224 X12940 M28393	(103-44.7)	M31951
Matrix metalloprotease 8 (neutrophil collagenase)	3.2 (1178/370)	J05556 NM002424	(1.0/-1.1)	
Calpain, small polypeptide	2.0 (4089/2059)	X04106 BC064998 BC023643 BC017308 NM001749 BC018931 BC000592 BC011903 BC007779 BC021933 BT009775 NM032330 BC006000 BC005397 AY052551	(1.1/1.3)	
Granzyme A	1.9 (1944/1022)	NM06144	———	

Table 1. Differentially expressed genes in LGL1 and LGL2. This data is based on Incyte Genomics and Affymetrix Chip FL 6800

	Incyte Genomics		Affymetrix	
Gene Name	BDE (p1/p2)	GenBankID	Fold Change (LGL1/LGL2)	GenBank ID If different from Incyte Genomics
		BC015739		
Caspase 8 (From RPA also)	1.4 (2035/1480)	U97075 NM033357 NM033358	(1.2/-1.4)	AF005775
Inducible or regulated proteins				
Interferon regulated factor 4	5.0 (1128/226)	U52682	(6/-1.5)	
TNF-α induced protein A 20	3.2 (1507/470)	M59465	(-1.3/-3.8)	
Heat shock 70 let protein 5 (Glucose regulated protein 78 kd)	2.8 (4090/1464)	X87949	(5.3/14.5)	M11717
RANTES (RPA also)	2.7 (2490/909)	M21121	(5.9/6)	
Human rap 2 mRNA for ras related protein	2.6 (899/327)	X12534	_____	
p53 inducible proteins	2.2 (2040/916)	L47738	(2.9/2.3)	
Glucose regulated proteins 58 kd receptors	2.2 (3661/1641)	AL043206	_____	
RECEPTORS				
CD8 antigen, alpha polypeptide (p32)	7.3 (4325/594)	M12824	(-1.2/-1.1)	M27161
Killer cell lectin-like receptor subfamily C, member 2 (NKG2-CII)	5.5 (2115/383)	AJ001684		
CD8 antigen beta	9.0 (1953/401)	NM004931	(7.2/5.2)	X13444

Table 1. Differentially expressed genes in LGL1 and LGL2. This data is based on Incyte Genomics and Affymetrix Chip FL 6800

	Incyte Genomics		Affymetrix	
Gene Name	BDE (p1/p2)	GenBankID	Fold Change (LGL1/LGL2)	GenBank ID If different from Incyte Genomics
polypeptide (p37)				
Musculin (activated B-cell factor-1)	4.1 (466/113)	AF087036		
Killer cell lectin-like receptor subfamily C, member 3 (NKG2-CII)	3.8 (1335/344)	AJ001685		
subfamily C, member 2 (NKG2-CII)	5.5 (2115/383)	AJ001684		
CD8 antigen beta polypeptide (p37)	4.9 (1953/401)	X13444	(7.2/5.2)	
Musculin (activated B-cell factor-1) Killer cell lectin-like receptor	4.1 (466/113)	AF060154		
Low affinity immunoglobulin Gamma FC receptor III-1 precursor	3.9 (1335/344)	J04162	(8.1/6.8)	
Filamin I (actin-binding protein-280)	3.8 (1085/287)	X53416	(2.1/1.9)	
Lectin-like Type II integral Membrane protein (NKG2-E)	3.8 (1300/344)	AJ001685		
Natural Killer cells group 7	3.1 (11251/3591)	S69115	(9.3/9.1)	
Protein tyrosine phosphatase type J receptor	2.1 (4614/2177)	L05148	(2.9/2.6)	

Table 1. Differentially expressed genes in LGL1 and LGL2. This data is based on Incyte Genomics and Affymetrix Chip FL 6800

	Incyte Genomics		Affymetrix	
Gene Name	BDE (p1/p2)	GenBankID	Fold Change (LGL1/LGL2)	GenBank ID If different from Incyte Genomics
Delta sleep inducing peptide Immunoreceptor	2.3 (5424/2319)	BE295817		
Lymphotoxin-Beta receptor precursor	2.3 (3587/1544)	AI271415		
MHC class II, DR beta 5 receptor	2.4 (2264/953)	X00700		
NKG2-D type II integral membrane protein	2.1 (1019/494)	X54870	(7.3/9.4)	
Protein tyrosine phosphatase Non-receptor type 12	2.1 (1036/494)	M93425	(1.6/1.0)	
Leukemia virus receptor (CGLVR1)	2.1 (713/340)	L20859	(2.9/2.5)	
Kinases and Phosphatases				
Dual specificity Phosphatase-1 (PAC-1)	4.2 (2484/585)	L11329	(1.6/1.2)	
Dual specificity Phosphatase-5	2.7 (857/320)	U10886	(1.1/1.6)	
Tyrosine protein tyrosine phosphatase	2.6 (713/272)	U15932	(1.2/2.3)	
Protein Kinase C etc	2.2 (2780/1239)	M55284	_____	
Zeta Chain (TCR) associated protein kinase (70kd)	2.1 (4614/2177)	L05148	(2.9/2.6)	

Table 1. Differentially expressed genes in LGL1 and LGL2. This data is based on Incyte Genomics and Affymetrix Chip FL 6800

	Incyte Genomics		Affymetrix	
Gene Name	BDE (p1/p2)	GenBankID	Fold Change (LGL1/LGL2)	GenBank ID If different from Incyte Genomics
Src Kinase-associated phosphoprotein of 55kd	2.1 (730/327)	Y11215	(3.3/2.4)	
Phosphatidyl inositol (4,5,bisphosphatase5-phosphatase homolog	2.1 (764/372)	638789	_____	
Protein phosphatase 2. Regulated subunit B (B56)	2.0 (1071/526)	U37352	(6.8/5.8)	
Protein Phosphatase 1, (catalytic subunit, alpha isoform)	2.0 (1643/835)	J04759		
Transcription Factors				
Runt related transcription factors 3	3.5 (2689/775)	D43968	(3.8/3.5)	
Miscellaneous				
EST.1	17.7 (346/189)	H06366		
EST.2	11.8 (2571/218)	AA482549		
EST.3	3.0 (544/182)	N47089		
Solute carrier protein	4.6 (785/172)	L14595	(1.4/1.6)	
Filamin A alpha	3.8 (1085/287)	X53416	(2.1/1.9)	
Hemoglobin delta	3.1 (2084/667)	V00505		
Hemoglobin beta	3.0 (4319/1419)	V00497		

Table 1. Differentially expressed genes in LGL1 and LGL2. This data is based on Incyte Genomics and Affymetrix Chip FL 6800

	Incyte Genomics		Affymetrix	
Gene Name	BDE (p1/p2)	GenBankID	Fold Change (LGL1/LGL2)	GenBank ID If different from Incyte Genomics
KIAA 0668 protein	2.6 (3476/1254)	AB014568		
MHC, Class II DR beta 3	2.4 (2264/953)	X00700		
PLECKSTRIN	2.4 (2033/854)	X07743	(2.0/2.4)	
Isocitrate dehydrogenase 2 (NADP+) Mitochondrial	2.2 (2067/893)	X69433	(2.2/2.7)	
Putative translation initiation factor	2.0 (4003/2046)	L26247	(-1.3/-1.5)	
Tubulin, Beta polypeptide	2.0 (2640/1349)	AW163523		
Ubiquitin B	1.9 (5668/3024)	BE250544		
Moesin	1.8 (5015/2750)	Z98946		
Nuclear factor of activated T cells, cytoplasmic	1.8 (2586/1440)	U85430	(1.8/2.9)	
Ubiquitin C	1.7 (3568/2071)	AA600188		
GTP binding protein, alpha 13	1.8 (2147/1195)	U87964	(-1.3/-1.5)	
Calritleulin Precursor	2.2 (3101/1384)	M84739	(2.0/2.2)	
KIAA0158 gene complete CDs	3.9 (2953/753)	063878		
Hemoglobin alpha I	3.2 (1074/333)	V00491		
T cell receptor	3.1 (987/315)	M30894	(5.0/11.3)	

Table 1. Differentially expressed genes in LGL1 and LGL2. This data is based on Incyte Genomics and Affymetrix Chip FL 6800

	Incyte Genomics		Affymetrix	
Gene Name	BDE (p1/p2)	GenBankID	Fold Change (LGL1/LGL2)	GenBank ID If different from Incyte Genomics
gamma chain				
FYN Oncogene related to SRC FGR, YES	3.x (3405/313)	Z97989		
EB1 mRNA	2.4 (1075/442)	U24166	(-1.8/-2)	
PLECKSTRIN	2.4 (2033/854)	X07743		
DNAJ protein Homolog	2.4 (237/1065)	D85429	(1.4/-1.7)	
MHC Class II HLA-DRW 10 beta	2.4 (2264/953)	D85429		
Lymphotoxin-beta receptor precursor	2.3 (3587/1544)	L04270		
Leucine Zipper Protein	2.3 (5424/2319)	50781	(1.4/-2.7)	
Probable protein disulfide Isomerase ER-60 precursor	2.2 (3661/1641)	Z49835	(1.4/1.0)	
Troponin T, Fast skeletal muscle Isomerase beta	2.2 (1628/743)	M21984		
Transforming growth factor receptor III	3.7 (764/204)	L07594	(10.6/7.1)	
DEC1, complete cds	3.5 (1498/1429)	AB004066		
Granulocyte Colony-stimulating Factor induced gene	3.1 (11251/3591)	S65115	(9.3/9.1)	

Table 1. Differentially expressed genes in LGL1 and LGL2. This data is based on Incyte Genomics and Affymetrix Chip FL 6800

	Incyte Genomics		Affymetrix	
Gene Name	BDE (p1/p2)	GenBankID	Fold Change (LGL1/LGL2)	GenBank ID If different from Incyte Genomics
Integrin, beta 2	2.7 (3718/1377)	M15395		
Clone 23912	2.6 (3476/1341)	AF038178		
Putative tumor suppressor Protein (RDA32)	2.5 (1145/453)	AF061836		
Down regulated genes				
Homo sapiens Indian hedgehog protein (IHH)	-18.6 (477/7779)	L38517	(-1.6/-1.1)	
CD20 Receptor	-16.2 (229/3703)	X07203	(1.1/-1.9)	
Human germline IgD chain gene, C-region	-11.0 (210/2313)	K02882	(-9.5/-7.5)	
Human transporter Protein (g17)	-10.4 (300/3124)	U49082	(-2/-1)	
Ribosomal protein S26	-6.2 (321/1853)	X69654	(-3.1/1.1)	
EST	-3.4 (429/1371)	R85437		
CD 72 antigen	-3.3 (353/1165)	M54992	(1.3/1.7)	
EST	-2.5 (629/1583)	AA916867		
Endothelial differentiation protein (Edg-1)	-2.5 (447/1033)	M31210	(-2.6/-5.2)	
Diacylglycerol kinase, alpha (80kD)	-2.5 (883/2172)	X62535	(-1.4/-2.3)	
60S Ribosomal	-2.3	Z12962	(-1.2/-1.2)	

Table 1. Differentially expressed genes in LGL1 and LGL2. This data is based on Incyte Genomics and Affymetrix Chip FL 6800

	Incyte Genomics		Affymetrix	
Gene Name	BDE (p1/p2)	GenBankID	Fold Change (LGL1/LGL2)	GenBank ID If different from Incyte Genomics
protein L41	(5372/2339)			
EST	-2.3 (708/1616)	AA134589		

Table 2. Genes upregulated in LGL1, LGL2 and LGL3/RA (Affymetrix U 95)

Gene Name	Accession No.	LGL1	LGL2	LGL3/RA	CD8+
		(Fold increase compared to Normal PBMC)			
perforin	32904_at	72.8	39.5	45.4	8.5
serine protease	40078_at	55.7	48.7	38.7	3.0
mast cell function-associated antigen homolog (MAFA)	34975_at	66.2	45.4	61.1	16.2
NK-receptor (NK-p46)	34039_at	53.6	45.2	50.6	7.8
gb=W28589	40913_at	47.7	41.2	44.2	23.9
suppressor related (DOC-1R)	35151_at	45.3	40.1	27.0	42.8
ribosomal protein S6 kinase 1 (RPS6KA1)	1127_at	42.4	40.0	50.6	2.2
butyrophillin (BT3.3)	38759_at	37.8	33.3	52.9	17.8
CD94	33531_at	35.2	34.2	17.9	7.3
MEGF9	36488_at	34.1	44.8	33.4	10.3
chronic granulomatous disease protein	40159_r_at	33.7	83.5	63.5	8.8
gamma2-adaptin (G2AD)	38799_at	30.3	29.2	27.5	40.5
calcineurin A2	39780_at	29.0	17.4	15.2	19.0
beta adaptin	35181_at	26.4	21.1	11.5	26.7
G protein-coupled receptor V28	40646_at	27.4	40.3	25.1	5.1
thrombin receptor	41700_at	22.5	8.3	14.2	4.8
GTPase-activating protein	36846_at	22.1	9.1	19.5	12.2
SH3 domain containing adaptor protein (SCAP	34432_at	21.9	10.4	22.8	10.3
AML1c	39421_at	21.5	17.2	31.7	10.6
KIAA0664 protein	34259_at	21.7	38.4	27.0	18.0
gb=AA978353	41126_at	21.4	8.8	13.9	1.4
Metk=megakaryocyte-associated tyrosine kinase	36264_at	20.7	17.1	13.1	1.4
vascular smoth muscle alpha-actin	32755_at	20.1	27.8	22.0	3.8
lysyl hydroxylase (PLOD)	36184_at	19.8	18.0	9.8	1.1
candidate tumor suppressor gene 21 protein isoform 1	40497_at	19.7	16.1	26.6	13.1

Table 2. Genes upregulated in LGL1, LGL2 and LGL3/RA (Affymetrix U 95)

Gene Name	Accession No.	LGL1	LGL2	LGL3/RA	CD8+
		(Fold increase compared to Normal PBMC)			
beta2-syntrophin (SNT B2)	40589_at	19.2	22.3	22.1	13.1
hexokinase III (HK3)	38372_at	18.8	39.6	4.1	6.7
telomeric repeat DNA-binding protein (PIN2)	1329_s_at	17.3	12.9	14.3	13.8
cytotoxic T-lymphocyte-associated serine esterase 1 (CTLA1)	32370_at	17.3	12.1	9.8	1.6
T cell specific protein (RANTES)	1404_r_at	17	10.2	18.3	4.5
CMRF-35-H9	41059_at	16.8	21.0	15.6	5.7
human immune interferon (IFN-gamma)	1021_at	16.7	21.7	18.8	-2.1
placenta (Diff48)	32978_g_at	16.5	14.4	6.9	23.7
medium-chain acyl-CoA dehydrogenase (MCAD)	37532_at	16.4	15.1	18.6	28.3
mRNA for Y3K1	40104_at	16.3	12.5	13.2	19.1
m3A methyltransferase (MT-A70)	32245_at	16.2	16.4	19.8	27.4
CD3G gene, exon 1	39228_at	16.2	6	5.3	3.4
PUTATUVE novel protein similar to many (archee)bacterial, worm and yeasy hypothetical proteins	41249_at	15.6	27.6	27.5	8.2
gb=A1004207	36732_at	15.8	25.1	17.6	22.2
microsomal glutathlone S-transferase (3-MGST3)	39018_at	15.6	21.8	16.9	28.4
similar to moise Choline/Ethanotamine Kinase (O55229)	32033_at	15.6	14.4	13.5	25.3
25S proteasome subunit p40.5	32211_at	15.3	15.2	11.7	12.7
Fo-gamma RIII-1	31499_at	15	5.8	5.4	-4.1
gb=AF070644	38852_at	14.6	16.6	14.4	8.4
gb=U79260	37242_at	14.5	15.9	11.5	17.5
Ste=20 related kinase SPAK	40986_at	14.5	10.9	18.2	8.2
guanine Nucleotide-Binding Protein Rap2	1819_at	14.5	5.8	6.5	4.1
SCA1 mRNA for ataxin	38142_at	14.2	13.2	16.9	7.7

Table 2. Genes upregulated in LGL1, LGL2 and LGL3/RA (Affymetrix U 95)

Gene Name	Accession No.	LGL1	LGL2	LGL3/RA	CD8+
		(Fold increase compared to Normal PBMC)			
butyrophillin (BTF4)	38760_at	14.2	13.3	18.7	7.1
HBV associate factor (XAP4)	32202_at	14.0	16.2	10.9	12.5
leukocystatin	34955_at	13.9	8.2	12.1	2.6
vav oncogene	1919_at	13.9	15.6	19.2	3.5
beta-2-adrenergic receptor	610_at	13.9	9.1	15.9	3.6
DNA from chromosome 19p13.2 coamide R31240, R30272 and R26549 comtaining the EKLF, GCDH, CRTC, and RAD23A genes	1751_g_at	13.9	17.2	10.9	23.2
DNA sequence from PAC 56H14 on chromosome 6q21-22. Contains FYN (P59-FYN, SYN, SLK) gene coding for two isoforms	40479_at	13.4	11.0	16.4	13.5
transcription factor LSF	40084_at	13.3	12.3	11.3	11.7
rap2	41318_g_at	13.2	3.3	5.9	2.8
activation (Act-2)	36674_at	12.8	7.1	12	-1.1
pM5	33414_at	12.8	10.2	8.8	8.2
CGAAT transcription binding factor subunit gamma	40488_at	12.8	18.3	18.7	14.6
CD4-related protein involved in lymphoma activation	36776_at	12.8	23.0	27.0	5.0
SYT Interacting protein SIP	41460_at	12.7	10.7	10.3	15.7
MHC class I	34934_at	12.6	13.9	18.2	21.2
DNA dependent ATPase and helicase (ATRX)	818_s_at	12.6	7.4	13.3	10.0
brutone tyrosine kinase (BTK), alpha-D-gelactosidase A (GLA), L44-like ribosomal protein (L44L) and FTP3 (FTP3)	36833_at	12.6	6.8	4	3.9
Natural killer cell BY55	33112_at	12.6	15.9	10	-2.2
leukocyte IgG receptor (Fc-gamma-R)	37200_at	12.5	9.8	9.7	-2

Table 2. Genes upregulated in LGL1, LGL2 and LGL3/RA (Affymetrix U 95)

Gene Name	Accession No.	LGL1	LGL2	LGL3/RA	CD8+
		(Fold increase compared to Normal PBMC)			
KIAA0080 gene	36144_at	12.4	14.3	11.8	3.0
tax1-binding protein TXBP181	499_at	12.4	11.1	17.0	6.6
gb=A1652860	41590_at	12.3	6.3	9.7	11.3
C-terminal binding protein 2	40780_at	12.1	5.5	5.5	1.1
NuMA	33822_at	11.9	19.8	9.8	25.0
	160043_at	11.9	13.1	3.1	4.2
lymphoma proprotein convertase (LPC)	34361_at	11.7	11.7	11.7	11.7
RGP3	37637_at	11.4	9.9	9.9	3
gb=W26655	39045_at	11.3	11.7	11.7	6.2
KIAA0064 gene	31802_at	11.3	3.8	3.8	17
KIAA0064 gene	37654_at	11.2	11.3	11.3	9.9
G9a	36200_at	11.1	11.0	11.0	6.7
Human transforming growth factor-beta type III receptor (TGF-beta)	1897_at	11.1	9	9	4.3
guanylate binding protein isoform 1 (GBP-2)	35735_at	11.1	29.5	29.5	6
KIAA0199 gene	37656_at	11.0	14.6	14.6	15.0
gb=AA194159	41282_a_at	10.9	10.7	10.7	17.7
carnitine palmitoyltransferase 1 type II	35936_g_at	10.9	11.8	11.8	8.8
carnitine palmitoyltransferase I type I	35228_at	10.8	14.3	14.3	7.9
Daxx	41151_at	10.8	15.4	15.4	13.7
B-ATF	39942_at	10.7	10.4	10.4	2.4
AUH	37616_at	10.7	16.0	16.0	10.5
(TAF1170-alpha)	37271_at	10.7	8.6	8.6	11.1
serine protease-like protein	37137_at	10.6	6.2	6.2	1.2
T-cell receptor T1 rearranged gamma-chain mRNA V-J-C region	41468_at	10.6	19	25.1	9.7
PEST phosphatase interacting protein homolog	34914_at	10.6	8.1	8.3	8.2

Table 2. Genes upregulated in LGL1, LGL2 and LGL3/RA (Affymetrix U 95)

Gene Name	Accession No.	LGL1	LGL2	LGL3/RA	CD8+
		(Fold increase compared to Normal PBMC)			
(H-PIP)					
KIAA0808 protein	33316_at	10.3	4.8	5.6	1.5
Nuclear protein, NP220	32674_at	10.3	7.5	12.1	15.3
beta-galatoside alpha-2,6-slalyltransferase	41352_at	10.2	8.9	6.1	13.8
HREV107-like protein	35704_at	10	8.9	5.4	-1.6
adenylyl cyclease type IX	33800_at	9.9	8.4	8.1	4.3
guanine nucleotide exchange factor mss4	38264_at	9.9	9.3	11.4	12.9
fibrinogen-like protein (pT49 protein)	39591_s_at	9.9	14	12.1	-3.1
XAP-5	36599_s_at	9.8	9.5	12.2	10.1
DNA from chromosome 19p13.2 cosmids R31240, R30272 and R23549 containing the EKLF, GCDH, CRTC, and RAD23A genes	1750_at	9.7	10.4	12.0	14.8
guanine nucleotide exchange factor	33280_at	9.6	6.9	6.2	4.7
DEAD-box protein p72 (P72)	41260_at	9.4	14.0	87.2	23.5
calcium/calmodulin-dependent protein kinase II	32105_f_at	9.4	7.3	10.3	7.2
IFN-gamma	40702_at	9.3	11.7	9.4	-2.8
IL-17	36229_at	9.3	19.1	4.6	25.5
KIAA0122 gene	40070_at	9.3	4.1	10.4	5
NKG2D gene, exons 2-5	36777_at	9.3	8.7	8	12.6
alanyl-tRNA synthetase	36185_at	9.2	12.1	15.8	25.5
gb=AL080203	40451_at	9.1	13.2	10.2	11.5
gb=AA524058	34359_at	9	6.1	4.6	7.6
P-glycoprotein (PGY1)	1576_g_at	9.0	8.6	18.1	14.9
bcl-xL	34742_at	8.9	6.6	3.4	7.3
putative dianoyl-CoA isomerase (ECH1) gene	32756_at	8.9	12	11.8	10.9
KIAA0245 gene	40123_at	8.9	5.4	4.8	4.3

Table 2. Genes upregulated in LGL1, LGL2 and LGL3/RA (Affymetrix U 95)

Gene Name	Accession No.	LGL1	LGL2	LGL3/RA	CD8+
		(Fold increase compared to Normal PBMC)			
gb=AF070533	41744_at	8.8	7.8	7.7	8.7
alpha-2,3-slayltransferase (SIAT4A)	40290_f_at	8.8	7.7	10.2	10.2
ADP-ribosylation factor	36193_at	8.8	9/1	9.1	11.7
gb=AI540958	34891_at	8.8	12.6	10.1	8.4
oligo A synthetase E	38388_at	8.8	7.8	16.8	1.2
gb=AA631972	39119_s_at	8.7	9.8	7.1	4.5
pyruvate dehydrogenase (EC 1.2.4.1) beta subunit	39160_at	8.7	4	6.2	6.2
gb=A1432401	39593_at	8.7	19.2	20.2	-6.9
gb=U51712	39698_at	8.6	9.6	3.3	3.9
glucocarebrosidase (GCB)	32632_g_at	8.6	10.3	8.3	7.5
T cell-specific protein (RANTES)	1405_1_at	8.6	8.1	9.4	4.9
aminoacytase-1 (ACY1)	37713_at	8.6	9.0	5.6	9.7
multidrug resistance protein 5 (MRP5)	1933_g_at	8.4	9.4	5.4	3.4
gb=AL050259	40521_at	8.2	7.3	10.7	7.5
carboxyl methyltransferase	37736_at	8.2	9.6	6.4	10.1
gb=AA176780	40485_at	8.2	15.9	10.2	21.7
KIAA0955 protein	41100_at	8.2	8.1	11.1	10.8
gb=AL079277	41710_at	8.1	7.7	3.1	-1.7
KIAA0129 gene	33253_at	8.1	11.1	7.4	10.6
gb=AA16987	39162_at	8.0	11.1	7.3	14.8
testle-specific cAMP-dependent protein kinase catalytic subunit (C-beta isoform)	36215_at	7.9	5.1	5.9	7.3
K1AA0898 protein	33107_at	7.8	4.5	7.9	6.1
tactile protein	34961_at	7.8	8.5	5.4	28.1
3-alkyladenine DNA glycosylase (HAAG)	37768_at	7.8	6.3	8.7	9.8
helicase-like protein (HLP)	37998_at	7.8	9.0	9.2	11.8

Table 2. Genes upregulated in LGL1, LGL2 and LGL3/RA (Affymetrix U 95)

Gene Name	Accession No.	LGL1	LGL2	LGL3/RA	CD8+
		(Fold increase compared to Normal PBMC)			
17-beta-hydroxysteroid dehyhydrogenase	36626_at	7.8	8.8	38.2	7.9
gb=AF035282	41679_at	7.7	6.7	6.8	3.8
beta2-chimaerin	33244_at	7.6	7.2	4.6	-1.5
Butyrophillin (BTF3)	38241_at	7.6	6.2	8.8	4.2
Protein kinase C-theta (PRKCT)	38949_at	7.6	6.1	8.8	7.5
homolog of yeast mutL (hPMS1) gene	525_g_at	7.5	8.9	9.0	9.1
heat shock protein (hsp 70)	1104_s_at	7.5	19.3	13.1	8.4
receptor protein 4-1BB	31540_at	7.5	7.4	6.7	-1.2
fibrinogen-like protein (pT49 protein)	39592_at	7.4	8.4	7.0	-1.6
RLIP76	36626_at	7.4	8.2	8.6	11.6
copper chaperone for auperoxide dismutase (CCS)	38088_at	7.3	7.8	10.5	9.3
TAR RNA binding protein 2 (TRBP2)	35657_at	7.3	7.3	5.5	7.3
N-myristoyltranferase 1	39000_at	7.3	10.0	10.0	13.8
gb=AA126515	41172_at	7.3	5.4	8.8	8.9
gb=W27519	32326_at	7.3	6	6.9	9.1
synaptogyrin 3	40314_at	7.2	7.4	9.7	3.4
gb=A1852521	39743_at	7.2	4.7	4.7	5.5
Human replication protein A	1382_at	7.2	4.0	4.8	6.9
puromycin sensitive aminopeptidase	39431_at	7.2	5.2	15.4	9.9
gb=A1014538	38623_at	7.2	7.9	7.2	9.9
gb=AF055004	34831_at	7.2	8.5	6.9	3.6
Endothelial Cell Growth Factor 1	1665_s_at	7.2	28.7	32.9	-11.3
gb=AL040137	41807_at	7.2	7.3	8.7	4.1
gb=AF007155	40472_at	7.1	6.6	6.9	6.7
lymphoid phosphatase LyP1	38808_at	7.1	3.1	5.6	2.7
Hanukah factor serine	40757_at	7.1	6.1	4.6	1.3

Table 2. Genes upregulated in LGL1, LGL2 and LGL3/RA (Affymetrix U 95)

Gene Name	Accession No.	LGL1	LGL2	LGL3/RA	CD8+
		(Fold increase compared to Normal PBMC)			
protease (HuHF)	35789_at	7.1	5	5.4	1.1
TM7XN1	33223_at	7	6.1	4.9	2
gb=AB011133	1942_s_at	7.0	7.5	5.4	10.2
cyclin-dependent kinase 4 (CDK4)	38171_at	7.0	4.0	3.5	2.7
WD repeat protein HAN11	1403_s_at	7	5.7	6.8	3.4
T cell-specific protein (RANTES)	34158_at	7.0	7.0	11.8	10.4
KIAA0067 gene	34724_at	7.0	7.9	6.5	5.2
gb=AI670100	626_s_at	6.9	13.4	8.2	1.9
BRCA1, Rho7 and vatl genes, complete cds, and lpl35 gene	41446_f_at	6.9	7.2	13	3.3
gb=H68340	37278_at	6.9	4	8.1	2.7
RasGAP-related protein (IQGAP2)	36999_at	6.9	8.5	13.3	15.9
RBP2-retinoblastoma binding protein 2	37359_at	6.8	5.8	3.7	4.8
KIAA0102 gene	35840_at	6.8	17	5.9	4.5
gb=AL050060	646_s_at	6.8	9.5	11.5	13.8
Clk2	32768_at	6.7	5.3	7.1	5.2
gb=AL048308	33854_at	6.7	7.3	9.2	5.7
gb=AA877795	38313_at	6.7	3.1	3.5	1.1
KIAA1062 protein	38464_at	6.7	6	6.9	9.9
a-glucosidase 1	40418_at	6.7	6.8	5.1	5.2
retinoblastoma	40485_at	6.7	8.2	8.9	8.3
gb=AF026402	32264_at	6.7	4.4	3.1	1.2
metase (MET-1)	33319_at	6.6	6.3	4	4.2
axin (AXIN)	35997_at	6.6	4.8	10.9	5.7
adenylate kinase (AK1)	514_at	6.6	5.4	11.4	13.6
cbl-b	40599_at	6.6	5.6	4.8	4.1
T-cell differentiation antigen Leu-2/T8					

Table 2. Genes upregulated in LGL1, LGL2 and LGL3/RA (Affymetrix U 95)

Gene Name	Accession No.	LGL1	LGL2	LGL3/RA	CD8+
		(Fold increase compared to Normal PBMC)			
gb=W26892	33850_at	6.5	7.8	6.5	8.9
mBA methyltransferase (MT-A70)	32246_g_at	6.5	6.7	8.5	13
1,4-alpha-glucan branching enzyme (HGBE)	32643_at	6.5	6.1	7.1	9.3
DP prostanoid receptor (PTGDR)	31782_at	6.4	6.7	3.6	4.3
interleukin 2 receptor gamma chain	1506_at	6.4	4.2	4.1	4.1
translational inhibitor protein	32173_at	6.4	5.5	4.5	4.9
gb=AI800578	34728_g_at	6.4	7.7	9.2	8.1
tudor repear assciator with PCTAIRE 2	40852_at	6.4	7.0	7.7	6.8
gb=AL080111	34752_at	6.3	3.9	7.9	7.4
granulocyte colony-stimulating factor induced gene	37121_at	6.3	4.9	4.7	1.1
carboxyl terminal LIM domain protein (CLIM1)	38937_s_at	6.3	6.1	4.4	-1.6
gb=AF091084	35329_at	6.3	9.1	6.9	11.4
gb=AL041683	32662_at	6.3	4.7	4.3	5.2
gb=AA160055	40937_at	6.3	4.8	5.0	12.5
NK receptor (NKp45), isoform d	34040_s_at	6.3	6.3	7.4	3.6
serine/threonine protein kinase EMK	965_at	6.3	6.9	6.1	8.7
small GTP-binding protein	40889_at	6.3	5.1	5.4	2.3
gb=AA576724	41648_at	6.3	5.8	6.4	5.6
RING zinc finger protein (RZF)	35811_at	6.3	6	8.5	4.7
KIAA0010 gene	32044_at	6.2	7.1	6.3	7.2
TBP-associated factor (hTAFII130)	142_at	6.2	5.7	5.8	6.8
gb=AW024285	41177_at	6.2	6.3	3.7	2.6
gb=D50920	34289_f_at	6.2	6.2	4.4	7.6
GARS-AIRS-GART	38384_at	6.2	7.3	8.6	7.5

Table 2. Genes upregulated in LGL1, LGL2 and LGL3/RA (Affymetrix U 95)

Gene Name	Accession No.	LGL1	LGL2	LGL3/RA	CD8+
		(Fold increase compared to Normal PBMC)			
SCA2	36998_s_at	6.2	6	7.4	9.5
sigma 3B	32030_at	6.1	4.6	6.7	1.5
KIAA0386 gene	37112_at	6.1	6.3	4.1	18.1
nucleolar protein hNop56	34882_at	6.1	5.5	4.2	11.4
RP105	40715_at	6.0	10.1	6.0	5.2
gb=W28167	34404_at	6.0	6.3	5.4	7.9
MAPidnase kinase 4 (MKK4)	36910_at	6.0	4.4	7.4	7.5
elF4GII	33907_at	5.9	5.9	7.5	2.6
WWp2-like mRNA	33629_at	5.9	6.1	5.3	2.9
G6PD gene for glucose-5-phosphate dehydrogenase	38043_at	5.9	3.5	4.8	9.0
LTG19	32400_at	5.9	6.2	6.3	5.4
KIAA0796 protein	38113_at	5.9	4.2	5.3	3.2
interleukin 2 receptor beta chain (p70-75)	1365_at	5.9	6	4.8	1.1
KIAA0060 gene	34332_at	5.8	7.8	7.9	14.5
low density lipoprotein receptor gene	32855_at	5.8	10.1	5.2	28.0
Huntington's Disease (HD)	37767_at	5.8	4.7	4.7	3.8
monocarboxylate transporter 2 (hMCT2)	35547_at	5.8	5.1	6	14.1
DNA from chromosome 19p13.2 cosmids R31240, R30272 and R28549 containign the ELKF, GCDH, CRTC, and RAD23A genes	1753_a_at	5.8	3.1	8.3	4.6
KIAA0053 gene	38149_at	5.8	5.2	9	5
Gb=AI143868	34816_at	5.8	4.6	5.1	7.7
serine phosphatase FCP1a (FCP1)	35979_at	5.8	6.2	5.4	5.2
similar to cytoplasmic dynain light chain 1	31655_at	5.7	7.7	6.0	3.2
KIAA1064 protein	36880_at	5.7	5.2	3.1	5.9

Table 2. Genes upregulated in LGL1, LGL2 and LGL3/RA (Affymetrix U 95)

Gene Name	Accession No.	LGL1	LGL2	LGL3/RA	CD8+
		(Fold increase compared to Normal PBMC)			
transactivator protein (CREB)	37535_at	5.7	5.8	8.6	10.2
Human immune interferon (IFN-gamma)	1611_s_at	5.7	5.3	4.5	-1
gb=AF052135	39391_at	5.7	8	7.6	9.6
aclyphosphatase, erythrocyte (CT) isoenzyme	33334_at	5.6	4.9	5.5	7.5
hRIF beta subunit (p102 protein)	33252_at	5.6	6.0	4.2	5.2
ABC transporter MOAT-C (MOAT-C)	41428_at	5.6	6.9	8.3	9.1
ras GTPase-activating-like protein (IQGAP1)	1825_at	5.6	6.2	6.1	4.2
protein tyrosine phosphatase (PTPase-alpha)	1496_at	5.6	3.8	5.2	3
retinoblastoma susceptibility	2044_s_at	5.6	4.4	5.6	2.3
KIAA0877 protein	39021_at	5.6	5.3	4.5	4.5
translocation T(4:11) of ALL-1 gene to chromosome 4	1124_at	5.5	4	7.6	6.4
osteoclast stimulating factor mRNA	467_at	5.5	4.9	4.4	4.1
kinesin-like DNA binding protein	356_at	5.5	5.1	9.2	6.5
lkB kinase beta subunit	35960_at	5.5	4.1	5.4	9.3
gb=AW044624	41551_at	5.4	5	6.6	4.6
gb=AA127624	33865_at	5.4	3.8	4.6	6.5
RNA binding protein DEF-3	40869_at	5.4	6.0	6.8	6.7
Protein phosphatase 2A B alpha1 regulatory subunit	178_at	5.4	4.4	7.8	8.1
integrin beta-7 subunit	2019_s_at	5.4	5.9	3.8	5.3
cdc25+ homolog	1347_at	5.4	4.7	3.8	10.3
Ndr protein kinase	38217_at	5.3	4.3	7.7	7.2
KIAA0625 protein	40083_at	5.3	6.6	7.9	8
KIAA1012 protein	38002_at	5.3	6.5	8	8.3
protein phosphatase 2A Balpha1 regulatory subunit	40788_at	5.3	4.2	7.2	6.3

Table 2. Genes upregulated in LGL1, LGL2 and LGL3/RA (Affymetrix U 95)

Gene Name	Accession No.	LGL1	LGL2	LGL3/RA	CD8+
		(Fold increase compared to Normal PBMC)			
WD40 protein BING4	33250_at	5.3	4.0	3.4	5.5
serine kinase SRPK2	1213_at	5.3	3.3	7.7	2.2
interferon regulatory factor 3	371_at	5.3	4.3	5.7	5.9
nuclear localization signal containing protein deleted in Velo-Cardio-Facial syndrome (Nivcf)	32745_at	5.2	4.9	4.4	4.4
gb=D45288	35310_at	5.2	3.2	3.3	2.1
gb=AI695103	35993_s_at	5.2	7.4	6.3	8.6
gb=X95808	41046_s_at	5.2	5.7	8.3	11.3
endo/exonuclease Mre11 (MRE11A)	32870_g_at	5.2	4.3	6.9	6.3
beige protein homolog (chs)	35695_at	5.2	5	7.6	2.9
gb=AL049703	32212_at	5.1	5.2	4.0	6.4
leuoocyte vacuolar protein sorting	35779_at	5.1	8.4	6.3	6
programmed cell death-2/Rp8 homolog	855_at	5.1	7.3	4.3	7.8
malate dehydrogenase precursor (MDH) mRNA, nuclear gene encoding mitochondrial protein	39001_at	5.0	4.5	4.6	5.2
gb=AL049955	34347_at	5	3.3	5.5	7
gb=U37012	33132_at	5	16.8	3.4	7.2
gb=D82351	31671_at	5	3.9	4.2	3.2
uracil-DNA glycosylase	37688_s_at	5.0	3.5	5.9	5.5
KIAA0011 gene	36932_at	5.0	4.5	5.8	7.8
YL-1 protein (nuclear protein with DNA-binding ability)	33873_at	5	4.2	6.7	7.1
tRNA synthetase-like protein	34291_at	5	7	6	8.2
protein kinase C-binding protein RACK7	842_at	5.0	4.9	3.8	4.6
KIAA0312 gene	34372_at	5.0	3.7	6.7	4.7
SF2p33	36099_at	4.9	4.6	3.7	5.0
gb=AB014597	39380_at	4.9	3.5	3.7	4.3

Table 2. Genes upregulated in LGL1, LGL2 and LGL3/RA (Affymetrix U 95)

Gene Name	Accession No.	LGL1	LGL2	LGL3/RA	CD8+
		(Fold increase compared to Normal PBMC)			
gb=R59697	35140_at	4.9	4.1	4.6	6.4
gb=U36501	37354_at	4.9	5.2	3.4	5.4
ZBP-59 protein	41465_at	4.9	3.6	5.2	5.1
ribulose-5-phosphate-epimerase	37797_at	4.9	4.0	7.2	9.2
C2f	39397_at	4.9	5.1	4.9	6.6
GT335	41749_at	4.9	5	5.9	4.3
Human poly(ADP-ribose) synthetase	1287_at	4.9	6	4.4	7.5
KIAA0132 gene	35322_at	4.9	5.3	9.3	6.2
gb=AF052162	41176_at	4.8	4.4	3.4	1.7
class 1 histocompatibility antigen-like protein mRNA	34427_at	4.8	3.1	4.0	4.0
gb=AF060862	40352_at	4.8	3.9	3.3	2.6
G4 protein (G4 gene, located in the class III region of the major hostocompatibility complex	41053_at	4.8	6.1	4.7	8.2
putative mitochondrial outer membrane protein import receptor (hTOM)	34345_at	4.8	6.4	4.4	7.2
nitriliase (NIT1)	39735_at	4.8	3.8	7.5	7.1
gb=L13435	160024_at	4.8	5.7	3.1	6.7
gb=L13435	33126_at	4.8	4.1	6.5	5.8
Smg GDS-associated protein SMAP	40779_at	4.8	3.9	4.4	6.3
KIAA0854 protein	41503_at	4.7	3.4	4.3	4.2
gb=AA173896	34340_at	4.7	9.3	6.5	8
gb=AA975427	31738_at	4.7	4.1	4.1	4
gb=W27939	38658_s_at	4.7	3.6	3.9	4.3
Human translational initiation factor (eIF-2)	1154_at	4.7	5.3	4	2.9
NADP-dependent isocitrate dehydrogenase (IDH)	39023_at	4.7	8.9	12.6	5.8
hterochromatin protein p25	37304_at	4.7	4.6	5.7	5.7

Table 2. Genes upregulated in LGL1, LGL2 and LGL3/RA (Affymetrix U 95)

Gene Name	Accession No.	LGL1	LGL2	LGL3/RA	CD8+
		(Fold increase compared to Normal PBMC)			
mNA for small GTP-binding protein	37466_at	4.7	6.4	5.7	6.3
methyl-CpG-binding protein	34355_at	4.7	4.4	4.6	5.8
mRNA for imogen	40072_at	4.6	4.2	4.9	6.6
transcription factor NFATx4	40823_s_at	4.6	4.5	3.1	3.9
nexin 1 (SNX1)	36583_at	4.6	8.5	12.3	9.8
gb=U79282	32069_at	4.6	4.0	5.2	4.2
gb=A1760162	41058_g_at	4.6	7.3	6.0	8.6
gb=AA224832	39120_at	4.6	5.7	9.3	9.4
KIAA0648 protein	34353_at	4.6	3.1	5.1	6.4
gb=AB007889	37383_at	4.6	4	5.5	1.3
homolog of yeast mutL (hPM31)	41481_at	4.6	3.6	4.5	5.5
UDP-glucose dehydrogenase (UGDH)	35214_at	4.6	3.9	4	6.4
KIAA0560 protein	41712_at	4.5	4.2	5.3	6.8
gb=AL060390	31852_at	4.5	3.8	3.7	3.6
similar to Drosophilia ash2	35804_at	4.5	5.8	8.5	5.7
gb=A1928387	33225_at	4.5	4.5	4.8	5.4
SCM-1beta precursor	31498_g_at	4.5	25.9	8.2	5.7
putative glucosyltransferase	32051_at	4.5	4.6	3	5.7
retinoic acid receptor responder 3 (RARRES3)	33236_at	4.5	4.2	4.6	1.6
KIAA0350 gene	34661_at	4.5	5.4	3	5.1
CACCC box-binding protein	41466_s_at	4.5	3.1	4.3	3.9
mutator gene (hMSH2)	860_at	4.5	5.0	3.8	13.1
tyrosylprotein sulfotransferase-2	35172_at	4.5	5	4.2	3
DNA polymerase gamma	1014_at	4.4	3.5	4.6	4
DORA protein	34946_at	4.4	14.8	13.2	-3.0
gb=A1246728	37046_at	4.4	4.3	3.8	5.9

Table 2. Genes upregulated in LGL1, LGL2 and LGL3/RA (Affymetrix U 95)

Gene Name	Accession No.	LGL1	LGL2	LGL3/RA	CD8+
		(Fold increase compared to Normal PBMC)			
galactokinase (GK2)	37825_at	4.4	3.7	4.7	3.4
gb=AW051579	33191_at	4.4	4.2	3.6	4.5
Heat shook protein 70 testis variant	40656_at	4.4	5.0	4.1	5.9
gb=AA142942	33399_at	4.4	5.3	4.3	4.6
gb=U26710	35832_at	4.4	3.1	5.0	7.4
stress-activated protein kinase 4	33245_at	4.4	3.8	4.0	3.3
ST15	35234_at	4.3	3.3	3.9	6.2
villin-like protein	37123_at	4.3	3.4	4.1	3.6
gb=U79255	37677_at	4.3	3.2	4.7	2.5
gb=L13744	35975_at	4.3	3.4	5.9	8.3
gb=AL049701	34446_at	4.3	3.3	5.1	2
FIP2 alternatively translated	41743_1_at	4.3	4.3	4.9	4.3
NF-AT4c	40822_at	4.3	4.1	4.5	3.9
putative poly(ADP-ribosyl) transferase (PARPL)	37303_at	4.3	4.4	4.9	4.6
KIAA0373 gene	38135_at	4.3	3.8	5.4	5.8
gb=W26640	35357_at	4.3	4	3.4	9.3
SCM-1beta precursor	31495_at	4.2	31.5	8.8	8.1
gb=D87077	38892_at	4.2	3.9	5.1	4.1
mitochondrial RNA polymerase	40232_at	4.2	3.5	5.3	4.7
gb=AA780049	40615_at	4.2	4.1	5.4	3.2
gb=AA905543	38620_at	4.2	5.0	4.8	2.7
(AF1q)	36941_at	4.2	4.0	5.1	11.1
KIAA0018 gene	38658_at	4.2	5.9	3.1	4.7
platelet activating receptor homolog (H963)	31919_at	4.2	3.4	13.9	9.9
SET-binding protein (IEF SP 3521)	34990_at	4.2	4.3	6.2	1.3
transformation sensitive protein (IEF SSP 3521)	207_at	4.2	8.3	3.6	6.6

Table 2. Genes upregulated in LGL1, LGL2 and LGL3/RA (Affymetrix U 95)

Gene Name	Accession No.	LGL1	LGL2	LGL3/RA	CD8+
		(Fold increase compared to Normal PBMC)			
protein-tyrosine phosphatase	1480_g_at	4.2	4.2	6.4	4.1
(GalT3 (beta3-Galactosyltransferase))	35944_at	4.1	3.9	5.4	3.5
Arp2/3 protein complex subunit p16-Arc (Arc15)	38392_at	4.1	3.8	3.7	3.9
nuclear receptor co-repessor N-CoR	39722_at	4.1	5.1	6.1	4.2
gb=AA808981	38287_at	4.1	5.2	4.4	2.3
transcription factor ISGF-3	AFFX-HUMISGF3A/M97935_3_at	4.1	5.2	7.3	2.7
Jak2 kinase	37468_at	4.1	6.1	5.5	3.5
transcription factor ISGF-3	AFFX-HUMISGF3A/M97935_MA_at	4.1	3.9	6.9	1
21-activated protein kinase (Pak1)	1558_g_at	4.1	6.9	6.1	-1.5
gb=D79985	33889_s_at	4.1	3.8	4.6	7.1
gb=AB002347	39797_at	4.1	4.5	7.1	8.1
gb=D79998	34858_at	4.1	3.8	4.7	8.8
short form transcription factor C-MAF (c-maf)	41506_r_at	4.1	4.8	3.1	2.6
gb=AW051579	33192_g_at	4.1	5.2	5.7	5.8
lycosylphosphatidyl inositol-anchored protein GPI-80	34498_at	4.1	3.7	11.2	1.6
DNA helicase (RECQL)	34684_at	4.1	5.2	7	8.6
KIAA0838 protein	34719_at	4.1	4	6.2	7.4
SKAP55	38862_at	4.1	3.3	4.3	2.2
Sel-1 like mRNA	40689_at	4	3.4	3.6	3.4
c-myc binding protein	1904_at	4	5.3	3.4	4.1
T-cell receptor alpha chain C region	432_s_at	4	5.1	3	4.8
calcium activated neutral protease large subunit (muCANP, calpain, EC	33908_at	4	5.5	3.9	3.5

Table 2. Genes upregulated in LGL1, LGL2 and LGL3/RA (Affymetrix U 95)

Gene Name	Accession No.	LGL1	LGL2	LGL3/RA	CD8+
		(Fold increase compared to Normal PBMC)			
3.4.22.17)					
uridine disphosphoglucose pyrophosphorylase	37373_at	4	3.6	3.7	3.7
SH2D1A	38147_at	4	3.4	4.4	3.9
gb=AL035295	37119_at	4.0	3.4	6.4	5.3
gb=AF070595	38170_at	4.0	3.0	4.2	6.5
gb=H05692	35283_at	3.9	4.0	5.4	5.4
gb=AI540318	41234_at	3.9	3.5	5.5	3.4
gb=X79882	38064_at	3.9	4.7	3.3	2.3
GAP binding protein p62dok (DOK)	815_at	3.9	5.3	6.9	3.7
OPA-containing protein	40998_at	3.9	4	4.1	5.4
myogenic determining factor 3 (MYOD1)	33482_at	3.9	4.0	4.2	4.9
gb=AA293354	38981_at	3.9	6.2	3.7	5.7
gb=AF006083	35271_at	3.9	3.4	3.1	3.2
ICAM-2	38454_g_at	3.9	6.4	3	5.7
protein-tyrosine phosphatase	1459_at	3.9	3.2	5.9	3.7
T -lymphocyte specific protein tyrosine kinase p56lck (lck) abberant mRNA	33238_at	3.9	3.6	3.7	4.7
zinc finger protein	39261_at	3.9	4.0	6.7	7.4
KIAA0097 gene	37293_at	3.8	3.4	5.4	4.3
cytosalic acetoacatyl-coenyme A thiolase	34790_at	3.8	3.1	3.2	6.8
NF-AT4c	250_at	3.8	3	4	2.7
gb=X77744	32883_at	3.8	4	6.1	5.4
gb=Y08614	37729_at	3.8	3.9	4.5	3.8
transcription factor WSTF	32261_at	3.8	4.4	5	5.5
TATA-binding protein mRNA	41441_at	3.8	3.2	4.6	7.3
KIAA0543 protein	41077_at	3.8	4.6	5.5	12.7
lymphocyte-specific protein tyrosine kinase (lck)	2059_s_at	3.7	3.9	4.1	4.7

Table 2. Genes upregulated in LGL1, LGL2 and LGL3/RA (Affymetrix U 95)

Gene Name	Accession No.	LGL1	LGL2	LGL3/RA	CD8+
		(Fold increase compared to Normal PBMC)			
CHD5 protein	32777_at	3.7	3.3	6.7	5.4
KIAA0549	40084_at	3.7	4	3.3	4.9
leukemia associated gene 1	33791_at	3.7	5.4	3.1	3.9
DM33	37007_at	3.7	3.9	4.6	5.6
branched chain alpha-ketoacid dehydrogenasekinase precursor	32828_at	3.7	3.2	7.6	2.9
gb=AL022398	40720_at	3.7	3.6	3.1	5.4
KIAA0748 protein	41585_at	3.7	3.5	5.5	3.6
gb=AL050018	46875_at	3.7	5.2	3.2	4.8
gb=D25538	40585_at	3.7	4.3	3.8	1.9
gb=X84908	37392_at	3.7	3.9	5.9	2.9
/gb=X70478	36877_at	3.6	3.8	4.8	4.4
Interleukin1-beta converting enzyme isoform beta (IL1BCE)	39320_at	3.6	3.1	3.1	-1.8
Rad50	1533_at	3.6	3.7	3.7	3.6
snRNA activating protein complex 190kD subunit (SNAP190)	35092_at	3.6	3.9	3.9	6.4
gb=AI655015	39932_at	3.6	5	5	6.2
TGF-beta activated kinase 1a	36905_at	3.6	5.1	5.1	7
TAFII20	802_at	3.6	5.1	5.1	4.9
gb=AA203246	41821_at	3.6	4.8	4.8	4.2
KIAA0039 gene	37646_at	3.6	5.1	5.1	3.2
KIAA0494	41830_at	3.5	3.4	3.4	4.3
gb=AI547262	33875_at	3.5	3.3	3.3	2
gb=AC002310	40905_at	3.5	7.5	7.5	4.0
MHC class III HSP70-2 gene (HLA)	31692_at	3.5	5.1	5.1	4.3
T-cell surface antigen CD2 (T11)	40738_at	3.5	4.2	4.2	3.5

Table 2. Genes upregulated in LGL1, LGL2 and LGL3/RA (Affymetrix U 95)

Gene Name	Accession No.	LGL1	LGL2	LGL3/RA	CD8+
		(Fold increase compared to Normal PBMC)			
tob family	39286_at	3.5	5.9	5.9	5.8
phosphorlbosypyro-phosphate synthetase-associated protein 39	37338_at	3.5	4.3	4.3	6.9
P-selectin glycoprotein ligand (SELPLG)	37541_at	3.5	3.1	3.1	3.2
leupaxin	36062_at	3.5	4.7	4.7	5.5
KIAA0992 protein	41191_at	3.5	3.6	6.5	-1.5
gb=W22296	36957_at	3.4	3.1	3.4	3
proloporphyrinogen oxidase	37098_at	3.4	3.7	4.2	8.2
prolyl oligopeptidase	37950_at	3.4	3.6	4.7	2.4
Toll/interleukin-1 receptor-like protein 3 (TILS)	34473_at	3.4	4.0	7.2	2.4
class-1 MHC-restricted T cell associated molecule (CRTAM)	36389_at	3.3	11.8	9.4	12.5
meningioma-expressed antigen 6 (MEA6)	41615_at	3.3	4.3	5.4	6.6
HMED7 (MED7)	36648_at	3.3	3.1	5.1	6.9
acetyl-coenzyme A transporter	34666_at	3.3	3.1	4.4	3.7
KIAA0241 gene	39761_at	3.3	4.8	7.1	7.7
gb=U00948	32185_at	3.3	3.6	4.8	3.4
gb=X53390	38794_at	3.3	4	3.2	6.1
Kruppel-type zinc finger protein	35588_at	3.3	3.3	6.5	11.8
gb=AL050159	38717_at	3.3	5.5	4.2	-4.7
protein-tyrosine phosphatase 1C	794_at	3.3	5.4	3.2	1.1
DAP-kinase mRNA	40049_at	3.3	5.8	9.4	-2.1
KIAA1105 protein	33457_at	3.3	4.8	5.2	5.4
son-a	39097_at	3.3	3.5	4	4.6
neutral amino acid transporter B mRNA	41178_at	3.3	4.2	3.4	2.8
candidate tumor suppressor gene 21 protein isoform 1	40498_g_at	3.2	3	3.6	2.3

Table 2. Genes upregulated in LGL1, LGL2 and LGL3/RA (Affymetrix U 95)

Gene Name	Accession No.	LGL1	LGL2	LGL3/RA	CD8+
		(Fold increase compared to Normal PBMC)			
mRNA					
KIAA0453 protein	32743_at	3.2	3.0	4.5	6.6
gb=AL060133	41815_at	3.2	4.3	5.5	4.7
DMA, DMB, HLA-Z1, IPP2, LMP2, TAP1, LMP7, TAP2, DOB, DQB2 and RING8, 9, 13 and 14 genes	41184_at	3.2	3.5	3	2.2
2,4-dienoyl-CoA reductase gene	38104_at	3.2	4.8	3.4	3.3
gb=AF055024	31875_at	3.2	3.3	4.4	4.9
KIAA0088 gene	37306_at	3.2	7.9	11.6	-1.7
mitochondrial 3-oxoacyl-CoA thiolase	41530_at	3.2	4.2	3.2	2.5
replication protein A 70kDa	38481_at	3.2	3.2	3.1	4.6
Human Interferon-gamma induced protein (IFI 16) gene	1456_s_at	3.1	3.6	6	3.1
VHL binding protein-1 (VBP-1)	171_at	3.1	3.6	3	4.6
butyrophilin (BTF5)	32629_f_at	3.1	3.6	5.3	3
gb=A1966201	35787_at	3.1	4.3	5.1	7.1
gb=AL050275	39115_at	3.1	3.7	4.4	8.1
gb=AI478147	40653_at	3.1	4.1	4.8	1.7
gb=AB028960	40829_at	3	6.7	6.7	7.5
gb=AL049435	38510_at	3.0	4.5	9.0	1.2
gb=AL080115	39442_at	3	3.7	6.3	4.6
Human phosphatase 2A	924_s_at	3	3.8	3.2	5
WNT7a	35783_at	3	4.5	4.4	10.0
Skeletal muscle abundant protein	32655_s_at	3.0	3.2	6.0	7.7
Gb=R59608	41302_at	3	3.4	3.9	3.5
Gb=AF070590	40760_at	3.0	3.7	4.1	2.1
Phosphatidylinositol-4-phosphate 5-kinase type II beta	35741_at	3	3.8	3.9	5.4
KIAA0541 protein	41430_at	3	3.4	4.6	3.7

Table 2. Genes upregulated in LGL1, LGL2 and LGL3/RA (Affymetrix U 95)

Gene Name	Accession No.	LGL1	LGL2	LGL3/RA	CD8+
		(Fold increase compared to Normal PBMC)			
FIP2 alternatively translated	41742_s	3	3	3.3	3.2

Table 3. Genes that are downregulated in LGL leukemia patients when compared to normal (Affymetrix U 95)

Name of the Gene	Accession No.	LGL 1	LGL 2	LGL3/RA
1. KIAA0508	33581_at	-2.8	-24.8	-23.7
2. retinal short-chain dehydrogenase/reductase retSDR1	40782_at	-1.4	-17.1	-10
3. KIAA0414	41695_at	-2.7	-13.1	-8.6
4. hypothetical protein FLJ10097	40916_at	-1.3	-10.6	-8
5. KIAA0552	38248_at	1.9	-9.7	-11.7
6. integrin alpha 6 subunit	39753_at	-2.1	-9.4	-5.3
7. KIAA0172	37225_at	-2.2	-9.1	-8.6
8. two-handed zinc finger protein ZEB	33440_at	1.5	-7.9	-8.0
9. sterol-C5-desaturase	33421_s_at	-2.4	-7.6	-10.0
10. nuclear factor RIP140	40088_at	-2.2	-6.9	-4.6
11. SCML2 protein	38518_at	-2.1	-5.8	-5.3
12. receptor protein-tyrosine kinase (HEK8)	1606_at	3.5	-5.5	-4.8
13. hSGT1	33746_at	-2.9	-5.5	-5.4
14. gb=AL080144	35672_at	-2.4	-5	-7
15. Dr1-associated corepressor (DRP1)	39077_at	-1	-4.9	-14.7
16. collagen binding protein 2	39166_s_at	-2.5	-4.7	-7.4
17. CD44 isoform RC (CD44)	31472_at	-2.3	-4.6	-4.6
18. USF2	38324_at	2.5	-4.5	-5.0
19. G protein-coupled receptor (EBI 1) gene exon 3	1097_s_at	3	-4.1	-5.4
20. serine/threonine kinase receptor-2-3 (SKR2-3)	34055_at	-2.2	-4.0	-3.9
21. gb=AC002073	36231_at	-2.2	-4	-12.8
22. nel-related protein 2	32598_at	4.1	-3.9	-5.3
23. transducin-like enhancer protein (TLE3)	38234_at	-2.4	-3.9	-3.2
24. DNA binding protein (SATB1)	38899_at	1.5	-3.8	-4.7
25. KIAA0443	37446_at	1.7	-3.8	-4.8
26. HSPNP	430_at	-1.2	-3.7	-3
27. GB=AF052160	34962_at	-1.7	-3.7	-9.6
28. LIM protein SLIMMER	32542_at	-1.1	-3.7	-4.8
29. calponin	40953_at	2.9	-3.7	-3.6

Table 4. Proteolytic Enzymes upregulated (data from the analysis of Incyte Genomics)

Gene Name	Balanced differential expression
Granzyme H	6.3
Cathepsin W (Lymphopain)	5.4
Perforin	3.8
Matrix metalloproteinase 8	3.2
Granzyme B precusor	3.1
Calpain, small polypeptide	2.0
Granzyme A	2.0
Caspase-8	1.4

Table 5. Proteolytic enzymes that are upregulated in leukemic LGL (data from the analysis of Affymetrix)

Name of the gene	Fold change compared to normal PBMC		
	CD8+	LGL1	LGL2
Granzyme H	2.2	28.6	14.7
Granzyme B	1.6	21.8	10.8
Perforin	7.6	10.3	44.7
Granzyme A	1.4	6.6	5.5
Cathepsin C	---	5.6	5.0

Table 6. Protease inhibitors that are downregulated in leukemic LGL (data from the analysis of Affymetrix)

Name of the gene	Fold change compared to normal PBMC		
	CD8+	LGL1	LGL2
Cystatin C	-97.5	-2.9	-1.4
Cystatin A	-20.5	-3.4	-1.5
α-1 Antitrypsin	-24.7	-2.5	-1.7
Metalloproteinase Inhibitor	-8.5	-4.8	-2.4

Table 7. Lymphokine/Chemokine profile of LGL leukemia sera*

Lymphokine/Chemokine	Elevated/ Total	Average Level (pg per ml)		Significance (P Value)
		LGL	Normal	
RANTES	26/27	17100	2890	<0.001
MIP-1α	5/27	1151	1051	=0.24
MIP-1β	16/27	2174	358	<0.001
IL-8	11/27	1097	405	<0.01
IL-1β	5/27	596	784	=0.39
IL-1Ra	9/27	479	143	<0.02
IL-18	16/27	561	134	<0.005
IFNγ	11/27	797	724	=0.26
TNFα	13/27	309	170	=0.11

* Findings from cytokine ELISAs are displayed. The pg/ml of each cytokine was determined using standards of known concentrations. P values as determined from grouped findings are shown.

References

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U.S. Patent Application Publication No. 2003/0190654

U.S. Patent Application Publication No. 2003/0032594

U.S. Patent Application Publication No. 2002/0120100

U.S. Patent Application Publication No. 2002/0035243

Altschul, S. F. *et al.* (1990) "Basic Local Alignment Search Tool" *J. Mol. Biol.* 215:402-410.

Altschul, S. F. *et al.* (1997) "Gapped BLAST and PSI-BLAST: A New Generation of Protein Database Search Programs" *Nucl. Acids Res.* 25:3389-3402.

Beltz, G. A., Jacobs, K. A., Eickbush, T. H., Cherbas, P. T., Kafatos, F. C. (1983) "Isolation of multigene families and determination of homologies by filter hybridization methods" *Methods of Enzymology*, R. Wu, L. Grossman and K. Moldave [eds.] Academic Press, New York 100:266-285.

Butz, E.A., Bevan, M.J. (1998) "Massive expansion of antigen-specific CD8+ T cells during an acute virus infection" *Immunity* 8:167-175.

Callan, M.F.C., Fazou, C., Yang, H., Rostron, T., Poon, K., Hatton, C., McMichael, A.J. (2000) "$CD8^+$T-cell selection, function and death in the primary immune response in vivo" *J Clin Invest* 106:1251-1261.

Crabtree, G.R., Clipstone, N.A. (1994) "Signal transmission between the plasma membrane and nucleus of T lymphocytes" *Ann Rev Biochem* 63:1045-1083.

Engler-Blum, G., Meier, M., Frank, J., Muller, G.A. (1993) "Reduction of background in problems in non-radioactive Northern blot analysis enables higher sensitivity than ^{32}P-based hybridizations" *Anal. Biochem* 210:235-244.

Grakoui, A, Bromley, S.K., Sumen, C., Davis, M.M., Shaw, A.S., Allen, P.M., Dustin, M.L. (1999) "The immunological synapse: a molecular machine controlling T-cell activation" *Science* 285:221-227.

Hoshino, S., Oshimi, K., Teramura, M., Mizoguchi, H. (1991) "Activation via the CD3 and CD16 pathway mediates interleukin-2-dependent autocrine proliferation of granular lymphocytes in patients with granular lymphocyte proliferative disorders" *Blood* 78:3232-3240.

Karlin S. and Altschul, S. F. (1990) "Methods for Assessing the Statistical Significance of Molecular Sequence Features by Using General Scoring Schemes" *Proc. Natl. Acad. Sci. USA* 87:2264-2268.

Karlin S. and Altschul, S. F. (1993) "Applications and Statistics for Multiple High-Scoring Segments in Molecular Sequences" *Proc. Natl. Acad. Sci. USA* 90:5873-5877.

Kasten-Sportes, C., Zaknoen, S., Steis, R.G., Chan, W.C., Winton, E.F., Waldmann, T.A. (1994) "T-cell receptor gene rearrangement in T-cell large granular leukocyte leukemia: preferential V alpha but diverse J alpha usage in one of five patients" *Blood* 83:767-775.

Kothapalli, R., Yoder, S.J., Mane, S., Loughran, T.P., Jr. (2002a) "Microarray results: how accurate are they?" *BMC Bioinformatics* 3:1-10.

Kothapalli, R., Kusmartseva, I., Loughran, M.P., Jr. (2002b) "Characterization of a human sphingosine-1-phosphate receptor gene (*S1P*$_5$) and its differential expression in LGL leukemia" *Biochimica et Biophysics Acta* 1579:117-123.

Kothapalli, R., Yoder, S.J., Kusmartseva, I.K., Loughran, T.P., Jr. (2003) "Characterization of a variant of PAC-1 in large granular lymphocyte leukemia" *Protein Expression & Purification* 32:52-60.

Lamy, T., Liu, J.H., Landowski, T.H., Dalton, W.S., Loughran, T.P., Jr. (1998) "Dysregulation of CD95/CD 95 ligand–apoptatic pathway in CD3+ large granular lymphocyte leukemia" *Blood* 92:4771- 4777.

Lamy, T., Loughran, T.P., Jr. (1999) "Current concepts: large granular lymphocyte leukemia" *Blood Rev* 13:230-240.

Loughran, T.P., Jr., Aprile, J.A., Ruscetti, F.W. (1990) "Anti-CD3 monoclonal antibody-mediated cytotoxicity occurs through an interleukin-2-independent pathway in CD3+ large granular lymphocytes" *Blood* 75:935-940.

Loughran, T.P., Jr. (1993) "Clonal diseases of large granular lymphocytes" *Blood* 82: 1-14.

McManus, Michael T. and Phillip A. Sharp (2002) "Gene Silencing in Mammals by Small Interfering RNAs" *Nature Reviews Genetics* 3:737-747.

Maniatis, T., E.F. Fritsch, J. Sambrook (1982) "Nuclease *Bal*31" *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Oshimi, K., Shinkai, Y., Okumura, K., Oshimi, Y., Mizoguchi, H. (1990) "Perforin gene expression in granular lymphocyte proliferative disorders" *Blood* 75:704-708.

Nagata, S., Golstein, P. (1995) "The Fas death factor" *Science* 267:1449-1456.

Sambrook, J., Fritsch, E.F., Maniatis, T. (1989) "Plasmid Vectors" In: Molecular Cloning. A Laboratory Manual., 2nd Edition., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 1-82.

Zambello, R., Trentin, L., Facco, M., Cerutti, A., Sancetta, R., Milani, A., Raimondi, R., Tassinari, C., Agostini, C., Semenzato, G. (1995) "Analysis of the T cell receptor in the lymphoproliferative disease of granular lymphocytes: superantigen activation of clonal CD3+ granular lymphocytes" *Cancer Res* 55:6140-6145.

Zimmermann, C., Rawiei, M., Blaser, C., Kaufmann, M., Pircher, H. (1996) "Homeostatic regulation of $CD8^+$ T cells after antigen challenge in the absence of Fas (CD95)" *Eur. J. Immunol.* 26:2903-2910.

Claims

We claim:

1. A method for screening for, detecting or diagnosing large granular lymphocyte (LGL) leukemia or an autoimmune disorder in a person or animal, said method comprising obtaining a biological sample from said person or animal, and screening for upregulated expression of a gene or genes, or a gene product thereof, whose expression is upregulated in LGL and/or screening for downregulated expression of a gene or genes, or a gene product thereof, whose expression is downregulated in LGL.

2. The method according to claim 1, wherein said gene or gene product whose expression is upregulated in LGL is selected from the group consisting of granzymes A, B, H, and K; cathepsin C and W; calpain small subunit; caspase-8; perforins; A 20; PAC-1; NGK2 receptors; RANTES; MIP-1alpha; MIP-1beta; IL-1 beta; IL-8; IL-1Ra; IFN-gamma; IL-18; IL-10; and IL-12 p35.

3. The method according to claim 1, wherein said gene or gene product whose expression is upregulated in LGL is selected from the group consisting of cystatin C and A; α-1 antitrypsin; and metalloproteinase inhibitor-8.

4. The method according to claim 1, wherein the expression of at least five, at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, or at least 40 genes or gene products whose upregulation is present in LGL is determined.

5. The method according to claim 1, wherein the expression of at least five, at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, or at least 40 genes or gene products whose downregulation is present in LGL is determined.

6. The method according to claim 1, wherein said biological sample is selected from the group consisting of bone marrow, lymph node, spleen, peripheral blood, lymph fluid, serous fluid, urine, and saliva.

7. A method for treating or preventing large granular lymphocyte (LGL) leukemia or an autoimmune disorder in a person or animal, said method comprising administering an effective amount of a composition that inhibits expression of a gene or genes, or that inhibits or blocks biological activity of a gene product, whose expression is upregulated in LGL and/or administering an effective amount of a composition that increases expression of a gene or genes, or that increases the amount of a gene product, whose expression is downregulated in LGL.

8. The method according to claim 7, wherein said gene or gene product whose expression is to be inhibited is selected from the group consisting of granzymes A, B, H, and K; cathepsin C and W; calpain small subunit; caspase-8; perforins; A 20; PAC-1; NGK2 receptors; RANTES; MIP-1alpha; MIP-1beta; IL-1 beta; IL-8; IL-1Ra; IFN-gamma; IL-18; IL-10; and IL-12 p35.

9. The method according to claim 7, wherein said gene or gene product whose expression is to be increased is selected from the group consisting of cystatin C and A; α-1 antitrypsin; and metalloproteinase inhibitor-8.

10. A composition for treating or preventing large granular lymphocyte (LGL) leukemia or an autoimmune disorder in a person or animal, said composition comprising a means for inhibiting expression of a gene, or inhibiting or blocking biological activity of a protein encoded by a gene, whose expression is upregulated in LGL.

11. The composition according to claim 10, wherein said composition comprises an antisense polynucleotide whose transcribed sequence is at least partially complementary to the transcribed sequence of a gene whose expression is upregulated in LGL, wherein expression of said gene is inhibited or blocked by expression of said antisense polynucleotide.

12. The composition according to claim 11, wherein said gene is selected from the group consisting of granzymes A, B, H, and K; cathepsin C and W; calpain small subunit; caspase-8; perforins; A 20; PAC-1; NGK2 receptors; RANTES; MIP-1alpha; MIP-1beta; IL-1 beta; IL-8; IL-1Ra; IFN-gamma; IL-18; IL-10; and IL-12 p35.

13. The composition according to claim 10, wherein said composition comprises an RNA interfering molecule.

14. The composition according to claim 13, wherein said RNA interfering molecule inhibits expression of a gene selected from the group consisting of granzymes A, B, H, and K; cathepsin C and W; calpain small subunit; caspase-8; perforins; A 20; PAC-1; NGK2 receptors; RANTES; MIP-1alpha; MIP-1beta; IL-1 beta; IL-8; IL-1Ra; IFN-gamma; IL-18; IL-10; and IL-12 p35.

15. The composition according to claim 13, wherein said RNA interfering molecule is a short interfering double-stranded RNA.

16. The composition according to claim 10, wherein said composition comprises:

a) an antibody, or an antigen binding fragment thereof, that specifically binds to said protein and blocks biological activity of said protein;

b) an antibody, or an antigen binding fragment thereof, that specifically binds to a receptor for said protein and prevents or inhibits binding of said protein to said receptor;

c) a peptide that binds to said protein or said receptor and block biological activity of said protein or said receptor; or

d) a combination of any of said antibody or peptide.

17. A composition for treating or preventing large granular lymphocyte (LGL) leukemia or an autoimmune disorder in a person or animal, said composition comprising a means for increasing expression or levels of a protein encoded by a gene whose expression is downregulated in LGL.

18. A method for screening for a compound useful in treating or preventing LGL or an autoimmune disorder in a person or animal, wherein said method comprises contacting an LGL cell with a test compound, isolating nucleic acid from said cell, and screening for: 1) inhibition of those gene sequences that are upregulated in LGL, or 2) increased expression of those gene sequences that are downregulated in LGL, or 3) both screening for inhibition of those gene sequences that are upregulated in LGL and screening for increased expression of those gene sequences that are downregulated in LGL are performed.

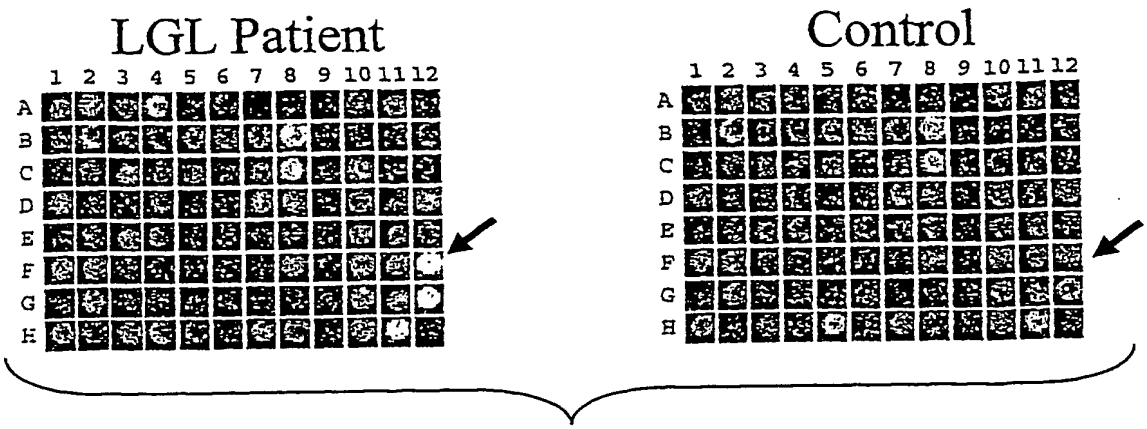


FIG. 1A

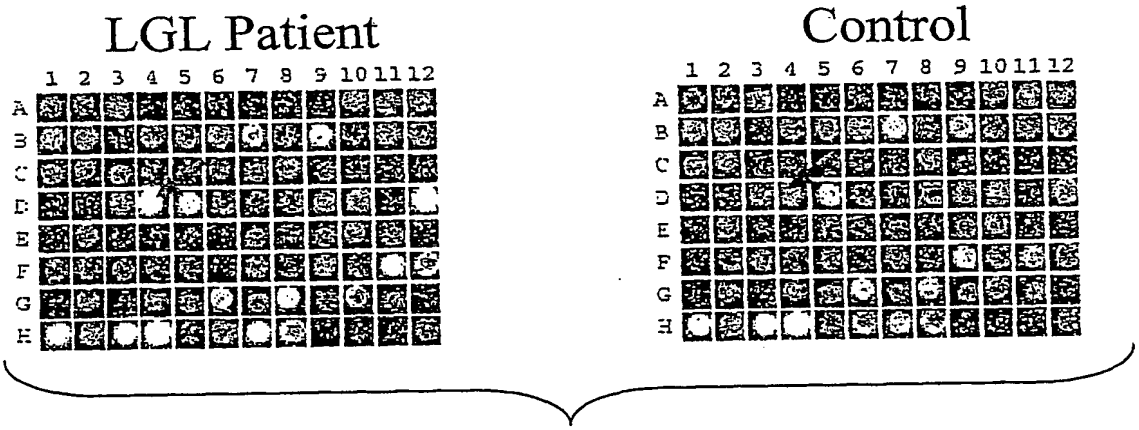


FIG. 1B

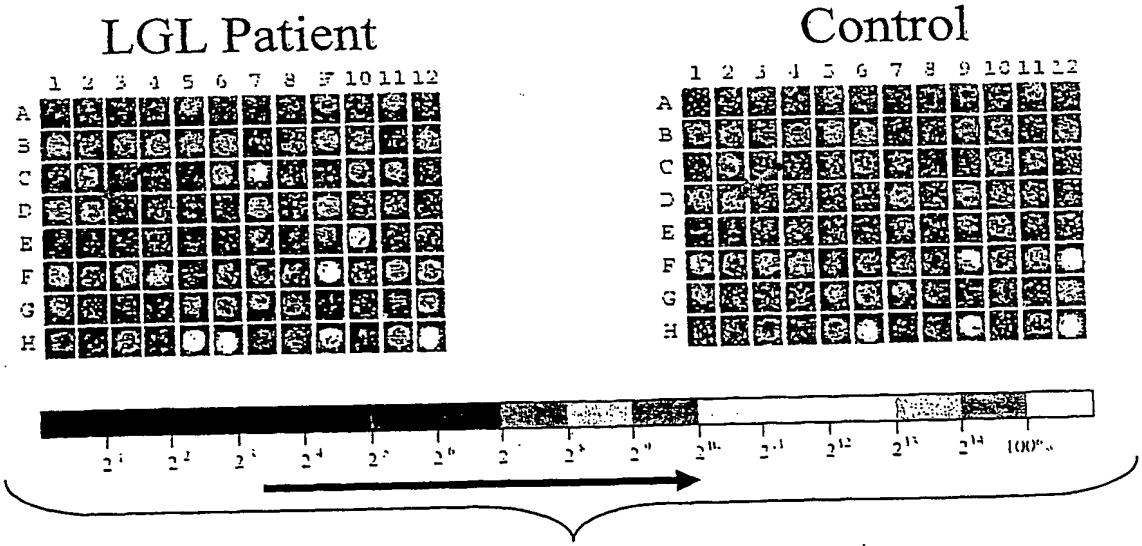


FIG. 1C

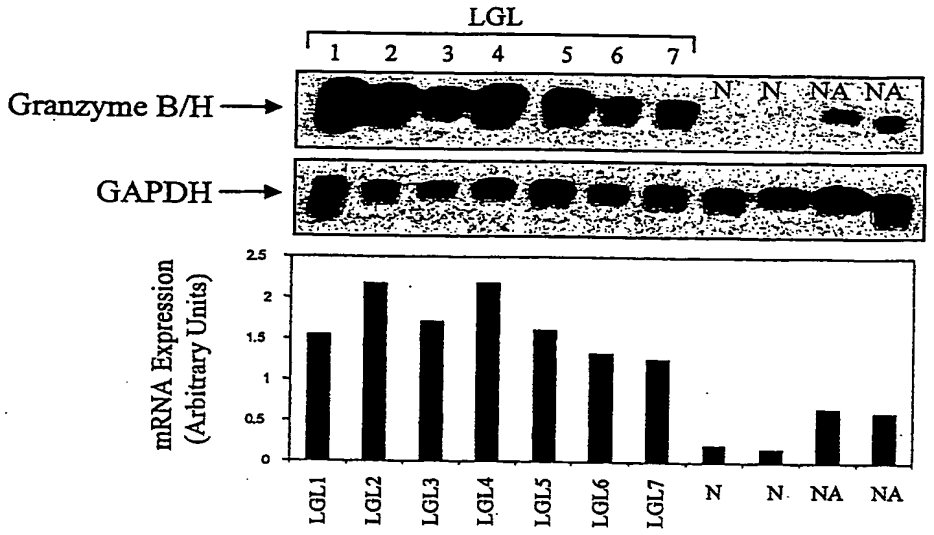

LGL
1 2 3 4 5 6 7
N N NA NA
Granzyme B/H
GAPDH
mRNA Expression (Arbitrary Units)
2.5
2
1.5
1
0.5
0
LGL1 LGL2 LGL3 LGL4 LGL5 LGL6 LGL7 N N NA NA

FIG. 2A

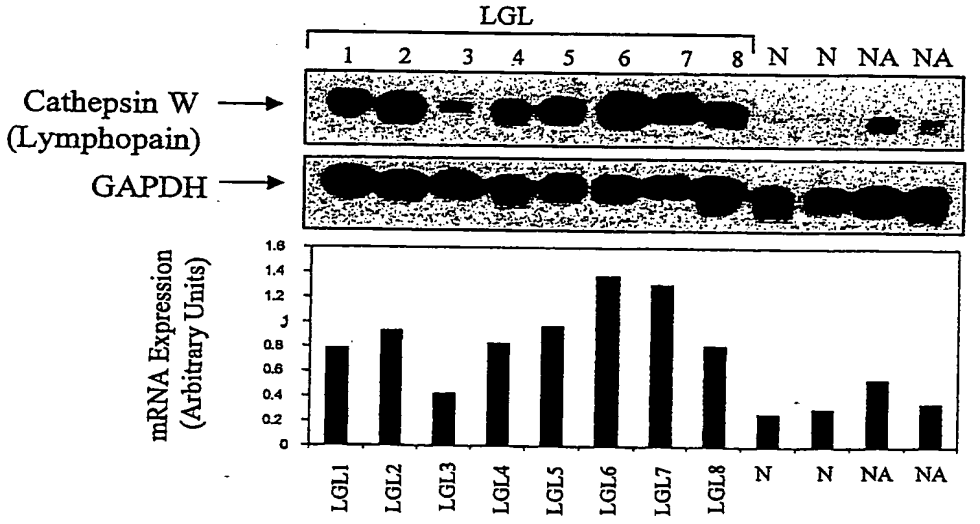

LGL
1 2 3 4 5 6 7 8 N N NA NA
Cathepsin W (Lymphopain)
GAPDH
mRNA Expression (Arbitrary Units)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
LGL1 LGL2 LGL3 LGL4 LGL5 LGL6 LGL7 LGL8 N N NA NA

FIG. 2B

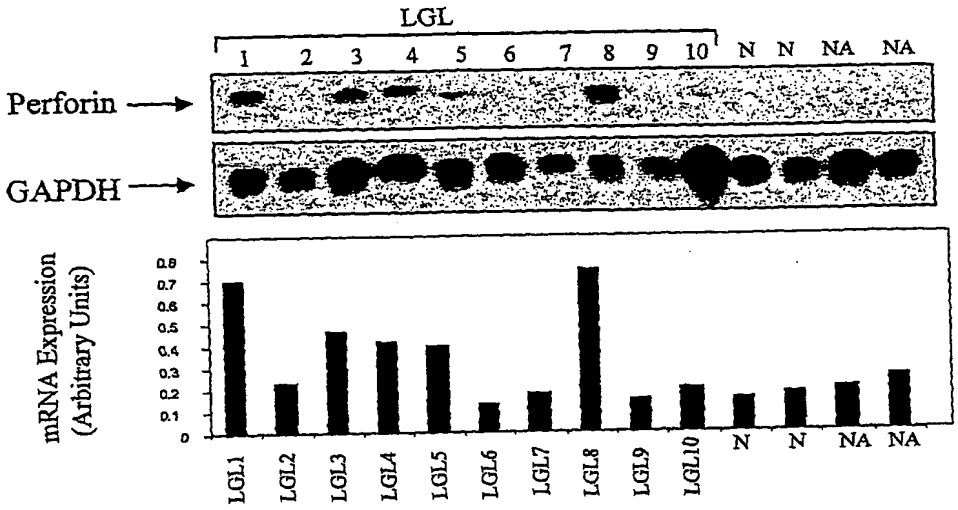

LGL
1 2 3 4 5 6 7 8 9 10 N N NA NA
Perforin
GAPDH
mRNA Expression (Arbitrary Units)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
LGL1 LGL2 LGL3 LGL4 LGL5 LGL6 LGL7 LGL8 LGL9 LGL10 N N NA NA

FIG. 2C

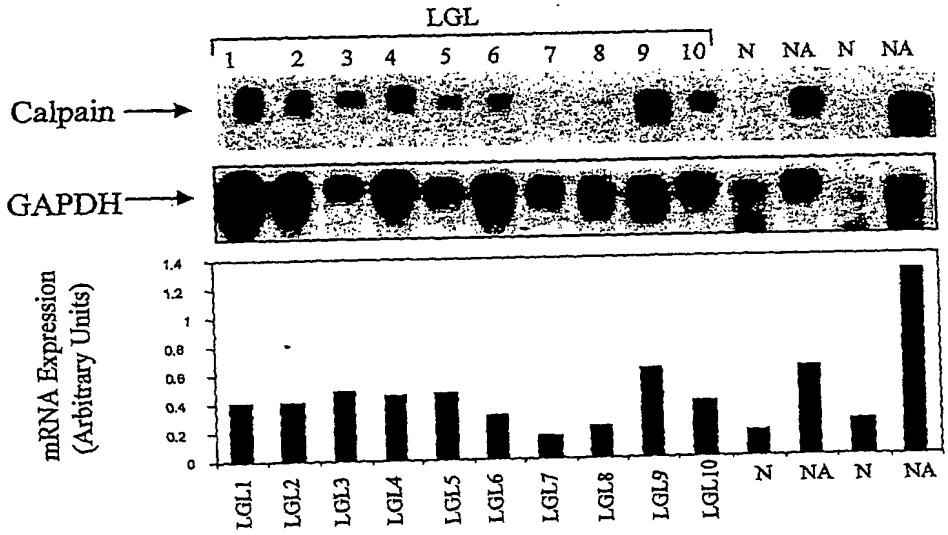

LGL
1 2 3 4 5 6 7 8 9 10 N NA N NA
Calpain
GAPDH
mRNA Expression (Arbitrary Units)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
LGL1 LGL2 LGL3 LGL4 LGL5 LGL6 LGL7 LGL8 LGL9 LGL10 N NA N NA

FIG. 2D

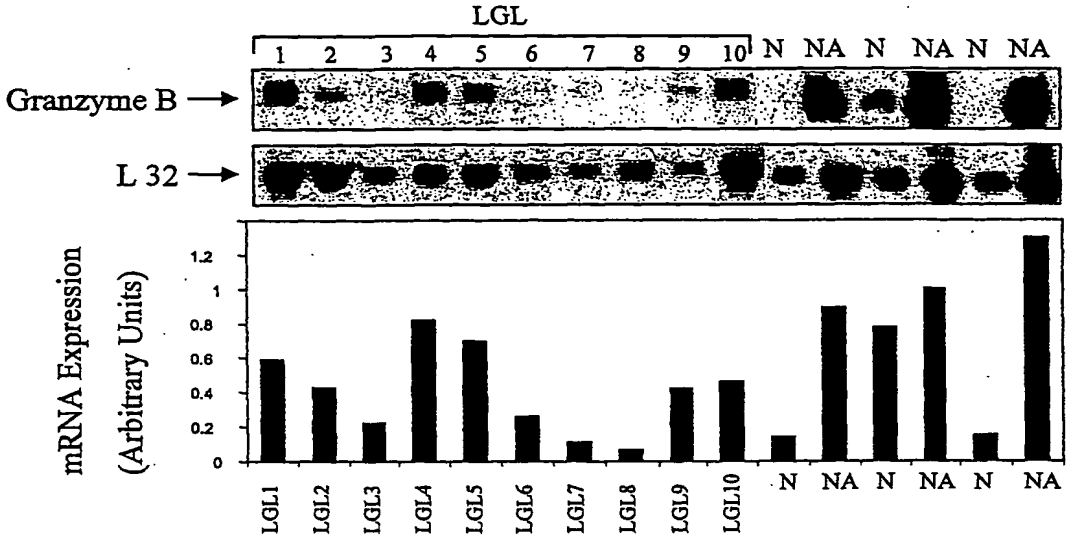

LGL
1 2 3 4 5 6 7 8 9 10 N NA N NA N NA
Granzyme B
L 32
mRNA Expression (Arbitrary Units)
1.2
1
0.8
0.6
0.4
0.2
0
LGL1 LGL2 LGL3 LGL4 LGL5 LGL6 LGL7 LGL8 LGL9 LGL10 N NA N NA N NA

FIG. 3A

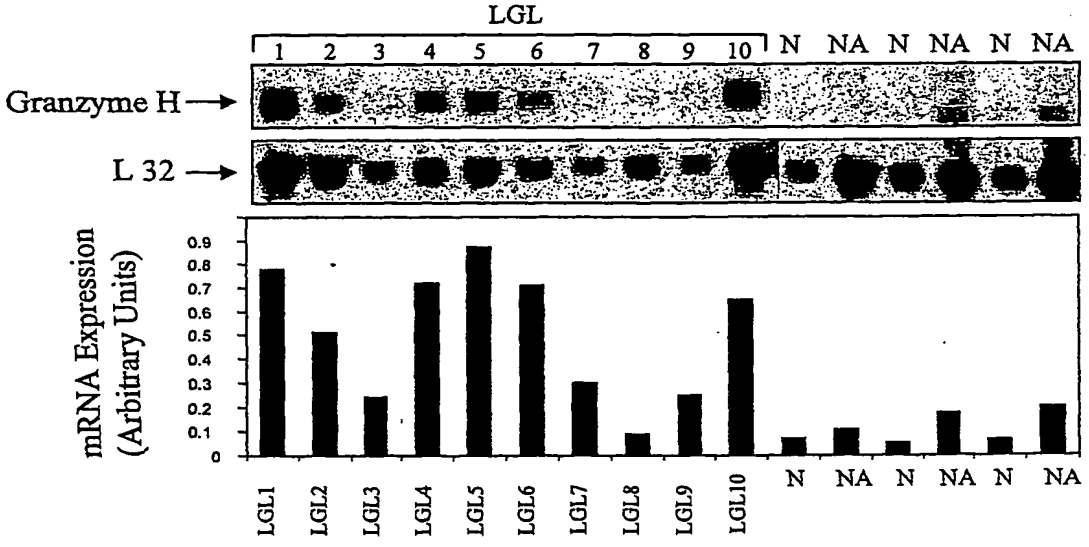

LGL
1 2 3 4 5 6 7 8 9 10 N NA N NA N NA
Granzyme H
L 32
mRNA Expression (Arbitrary Units)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
LGL1 LGL2 LGL3 LGL4 LGL5 LGL6 LGL7 LGL8 LGL9 LGL10 N NA N NA N NA

FIG. 3B

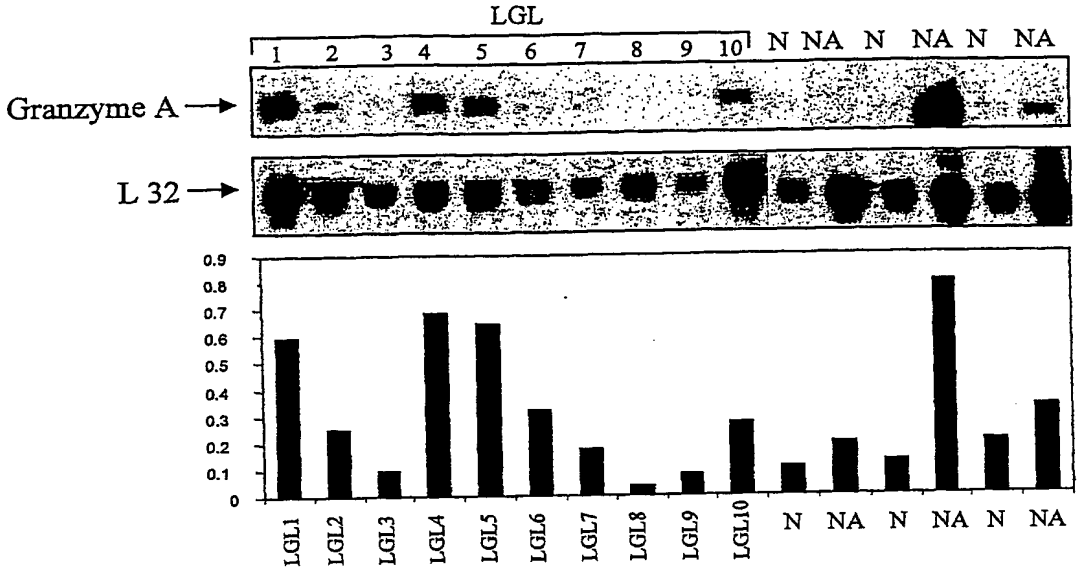

LGL
1 2 3 4 5 6 7 8 9 10 N NA N NA N NA
Granzyme A
L 32
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
LGL1 LGL2 LGL3 LGL4 LGL5 LGL6 LGL7 LGL8 LGL9 LGL10 N NA N NA N NA

FIG. 3C

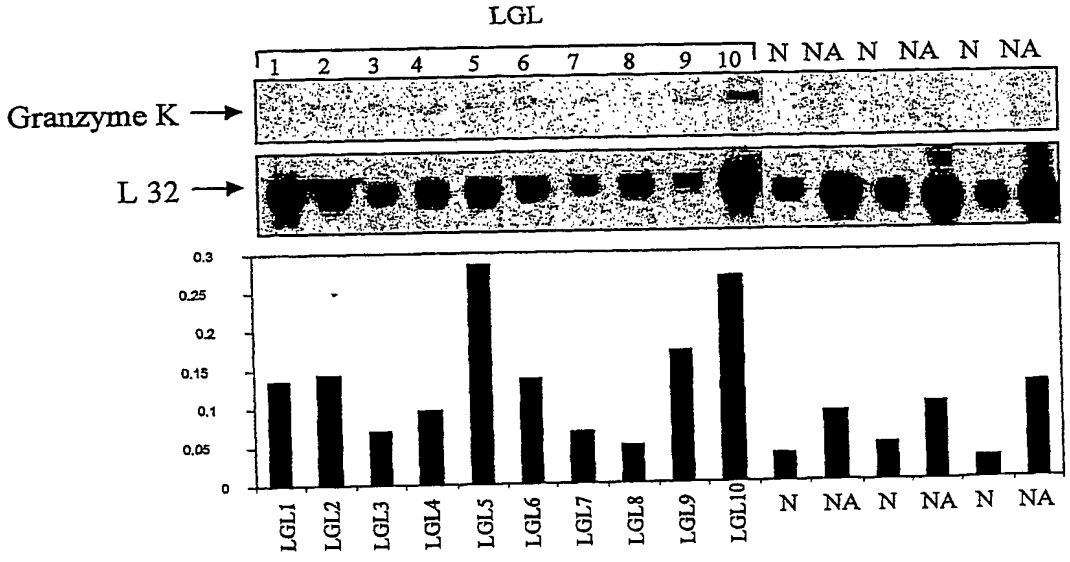

LGL
1 2 3 4 5 6 7 8 9 10 N NA N NA N NA
Granzyme K
L 32
0.3
0.25
0.2
0.15
0.1
0.05
0
LGL1 LGL2 LGL3 LGL4 LGL5 LGL6 LGL7 LGL8 LGL9 LGL10 N NA N NA N NA

FIG. 3D

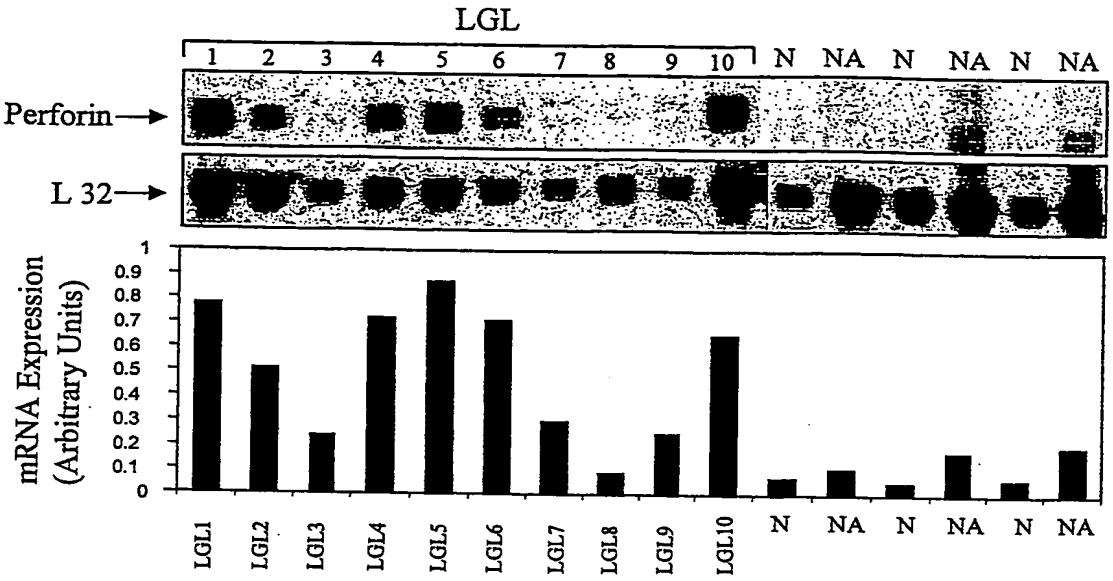

LGL
1 2 3 4 5 6 7 8 9 10 N NA N NA N NA
Perforin
L 32
mRNA Expression (Arbitrary Units)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
LGL1 LGL2 LGL3 LGL4 LGL5 LGL6 LGL7 LGL8 LGL9 LGL10 N NA N NA N NA

FIG. 3E

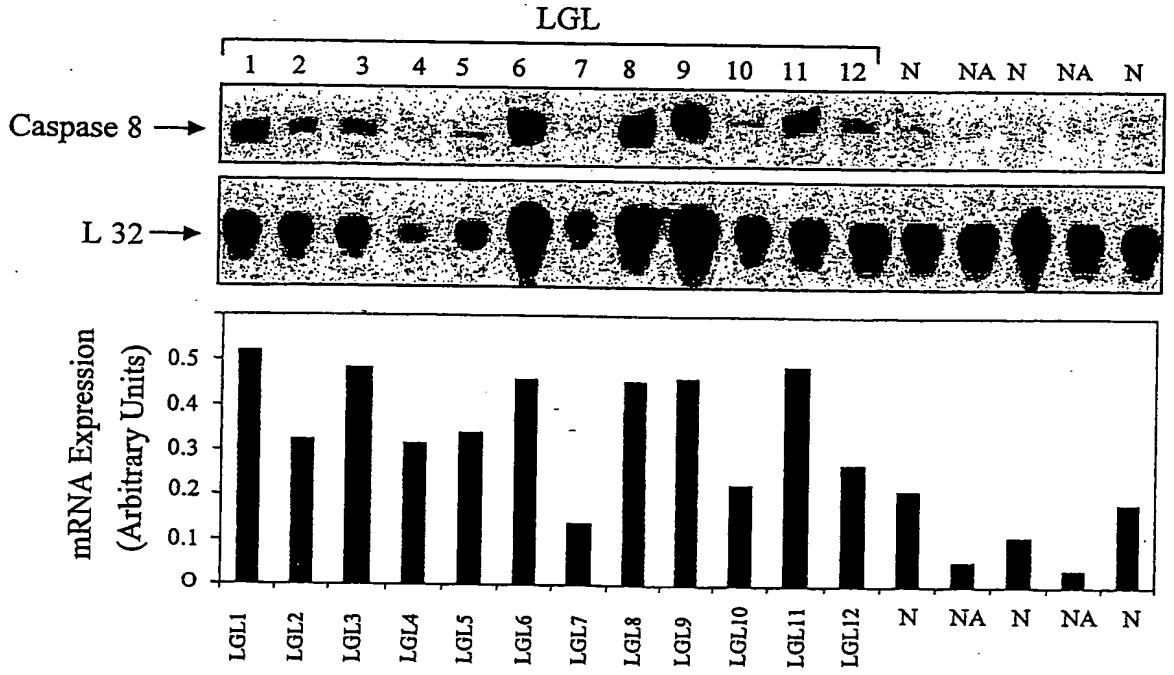

LGL
1 2 3 4 5 6 7 8 9 10 11 12 N NA N NA N
Caspase 8
L 32
mRNA Expression (Arbitrary Units)
0.5
0.4
0.3
0.2
0.1
0
LGL1 LGL2 LGL3 LGL4 LGL5 LGL6 LGL7 LGL8 LGL9 LGL10 LGL11 LGL12 N NA N NA N

FIG. 3F

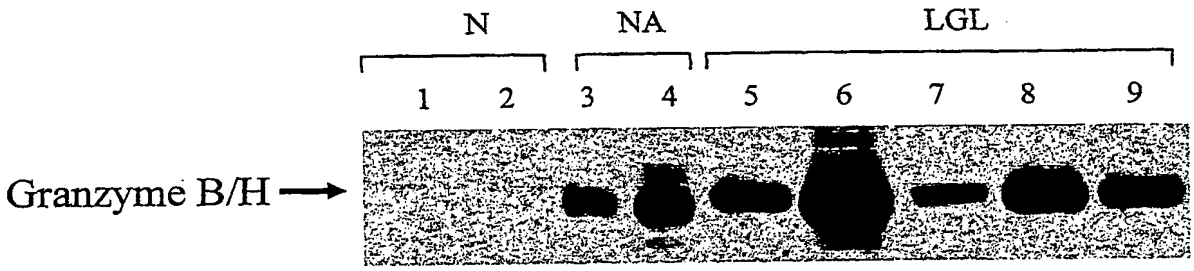


FIG. 4

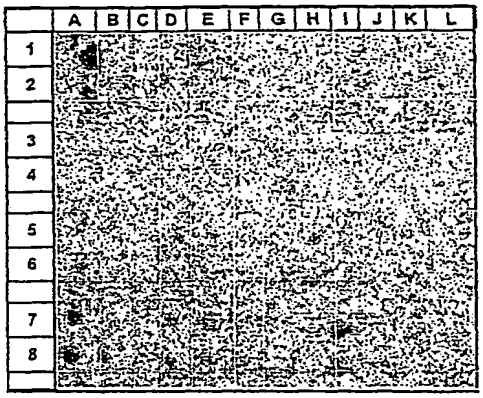


FIG. 5A

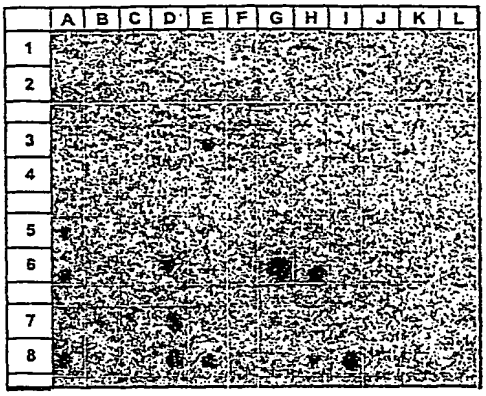
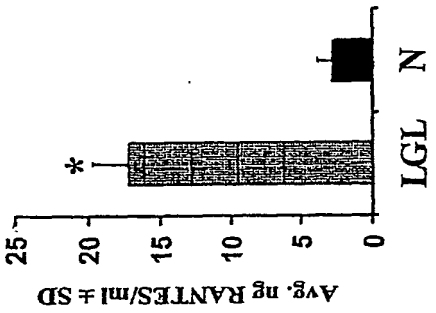


FIG. 5B

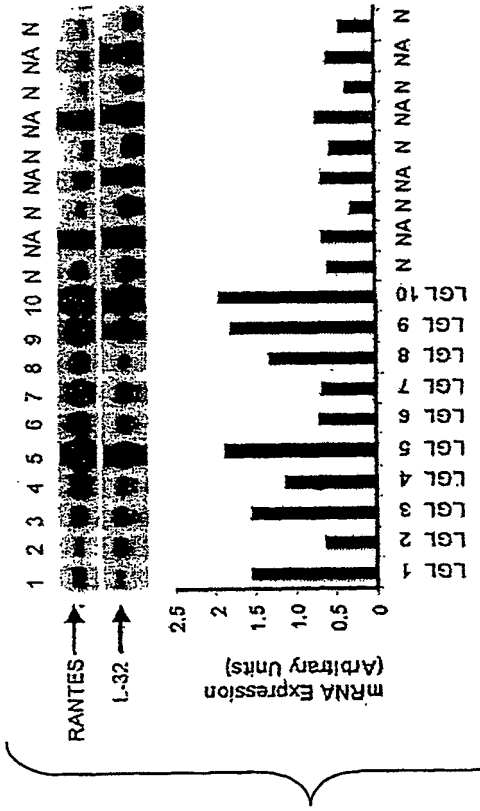
	A	B	C	D	E	F	G	H	I	J	K	L
1 2	Pos	Pos	Neg	Neg	ENA-78	GCSF	GM-CSF	GRO	GRO-α	I-309	IL-1α	IL-1β
3 4	IL-2	IL-3	IL-4	IL-5	IL-6	IL-7	IL-8	IL-10	IL-12	IL-13	IL-15	IFN-γ
5 6	MCP-1	MCP-2	MCP-3	MCSF	MDC	MIG	MIP-1β	MIP-1α	RANTES	SCF	SDF-1	TARC
7 8	TGF-β	TNF-α	TNF-β	EGF	IGF-1	Ang	OSM	Tpo	VEGF	PDGF-β	Leptin	Pos

FIG. 5C



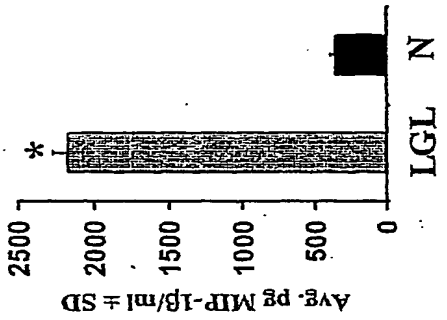
Sera

FIG. 6B



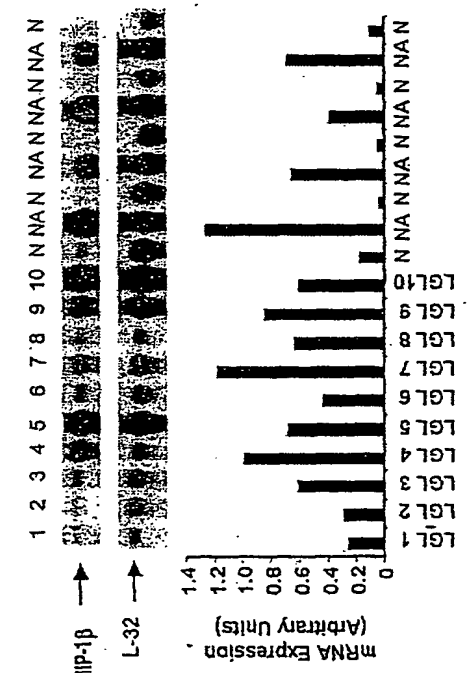
mRNA

FIG. 6A



Sera

FIG. 7B



mRNA

FIG. 7A

FIG. 8A

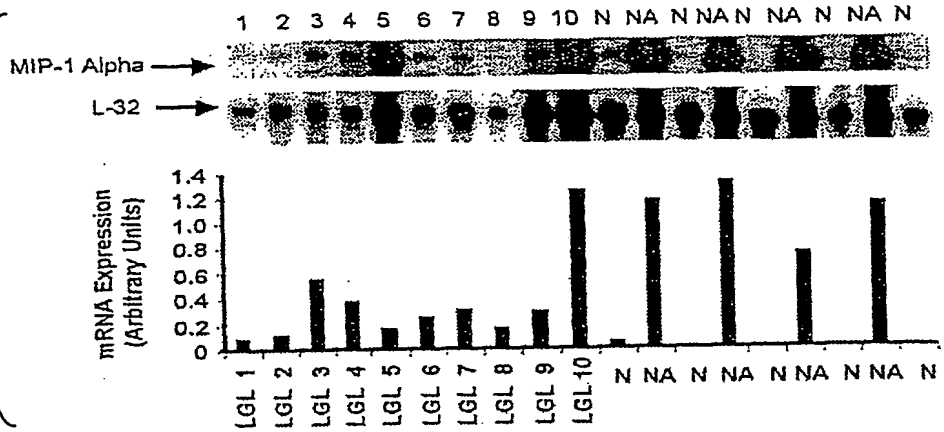


FIG. 8B

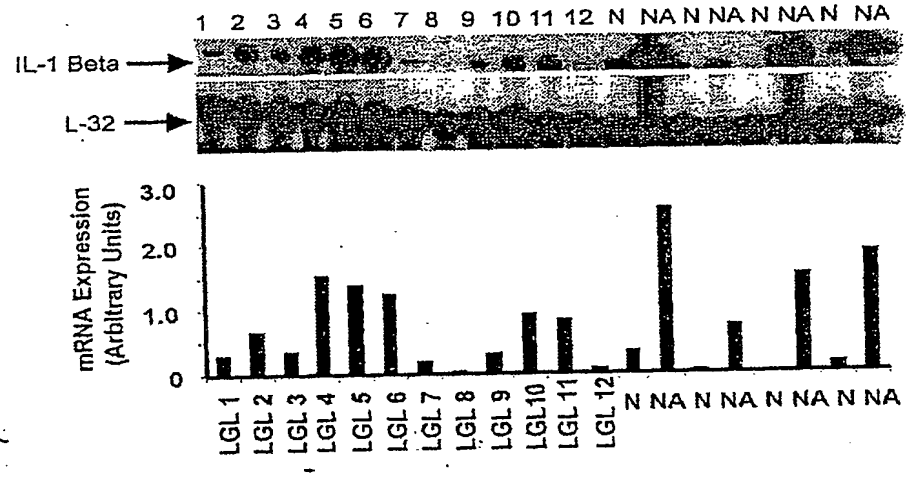


FIG. 8C

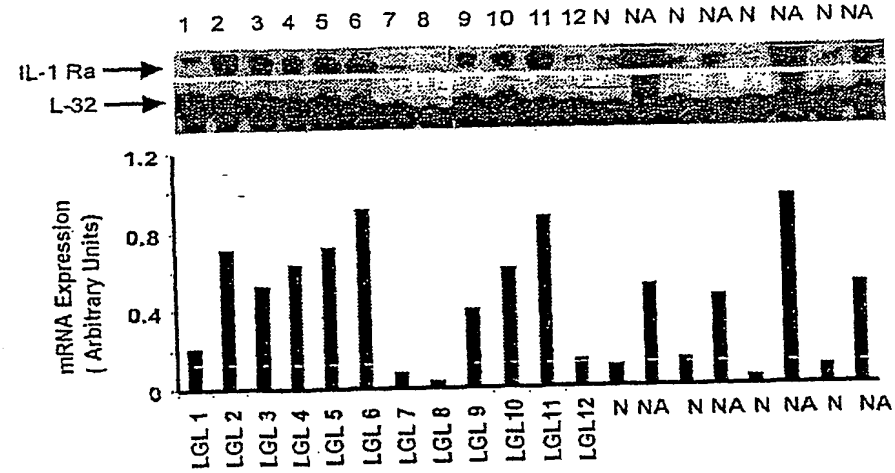


FIG. 9A

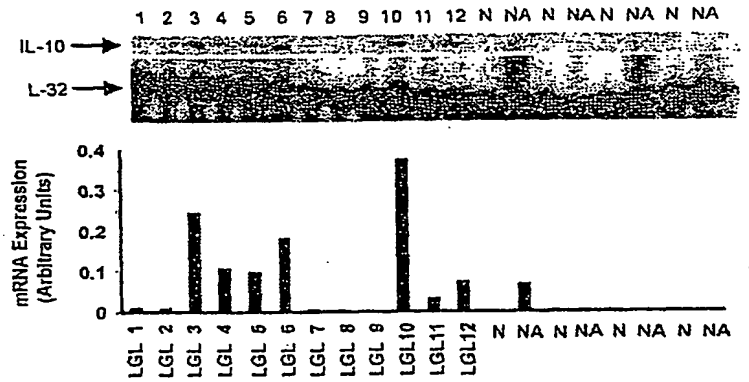


FIG. 9B

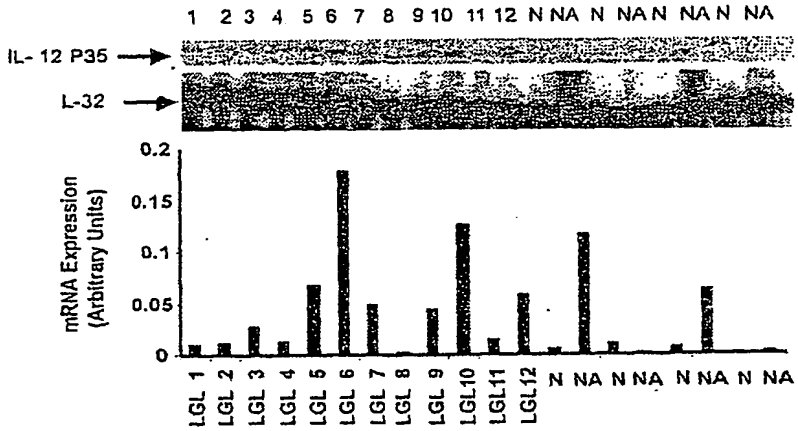


FIG. 9C

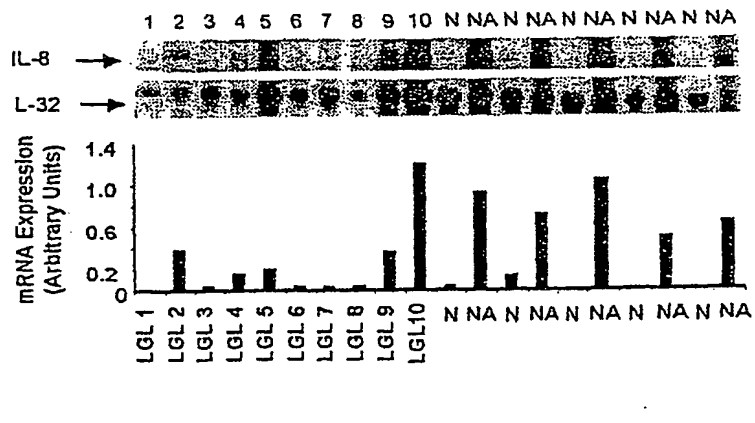


FIG. 10A

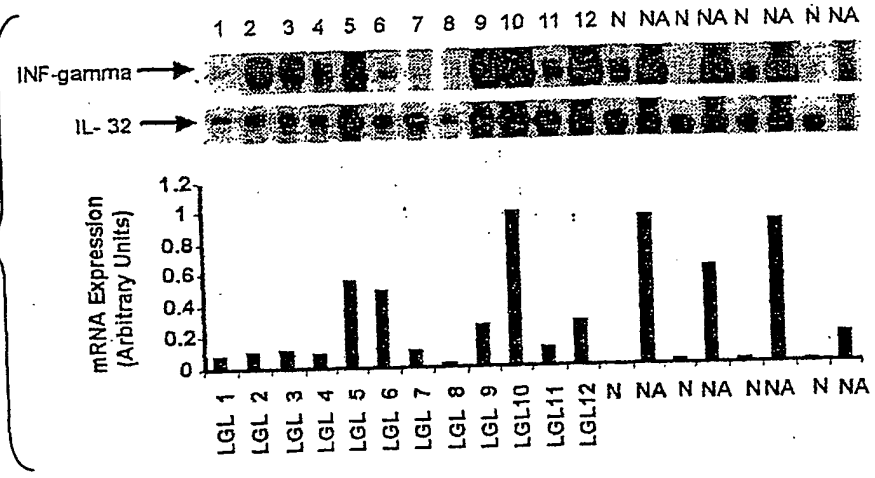


FIG. 10B